Final Report

Building a Bridge to the Ethanol Industry: Subcontract ZXE-9-18080-02

Corn Fiber Conversion in the Ethanol Industry

by

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The following is the final report for NREL Subcontract ZXE-9-18080-2, Corn Fiber Conversion to the Ethanol Industry. This report covers the period March 15, 1999 to January 31, 2000.

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Summary

The current fermentation alcohol industry in the US is based on utilization of glucose and/or starch derived from corn. Current annual production is in excess of 1.2 billion gallons. Corn wet-milling processes currently account for more than 60% of the fermentation ethanol capacity, with the balance reflecting various types of dry-milling or hybrid processes. Prospects for utilizing biomass materials in corn-to-ethanol facilities are improving with advances in cellulose pretreatment technologies; the development of microorganisms that can ferment arabinose, xylose and glucose; reduction in enzyme costs; and improvements in technology for pretreating cellulose. Improvements in enzyme-based cellulose conversion technologies have significant potential for processing fiber from a wet-milling process to ethanol. This fiber is generated as a co-product in corn wet-milling plants, and is currently sold as an animal feed supplement.

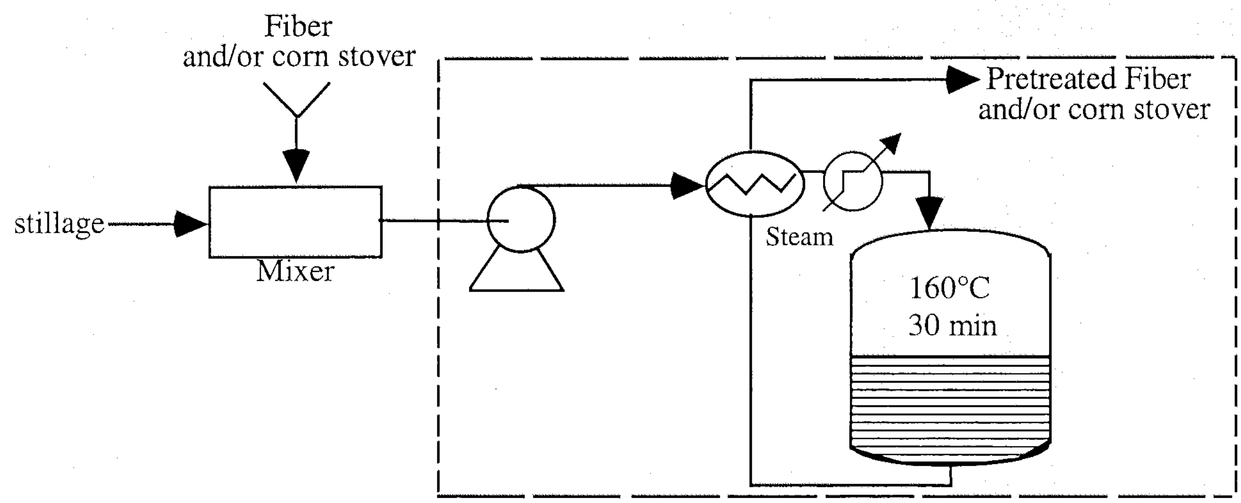
A three-way cooperative effort was initiated between Purdue University, Pekin Energy (now Williams Energy Services) (Mr. Gary Welch), and USDA NCAUR (Dr. Rod Bothast). This effort has performed preliminary tests of new technology for pretreating the corn fiber by pressure cooking it in water, followed by hydrolysis and pentose fermentation. The cooperative research demonstrated the viability of fiber pretreatment through pilot plant runs, and demonstrated the fermentability of the resulting hexoses and pentoses to ethanol by a recombinant microorganism. The next step is to scale-up this work for an in-plant trial, under the auspices of continued university-industry-government cooperation.

This subcontract addressed a study to generalize the combined results and experience of fiber processing to ethanol in the industry. This study carried out a design of a corn-fiber process based on aqueous pretreatment of the fiber; fermentation of either hexoses, or hexoses and pentoses to ethanol; and disposition of the remaining proteins and other streams resulting from such a process. Equipment design and process economics were specified for an enzyme-based hydrolysis process using the throughputs associated with the Williams wet-milling plant.

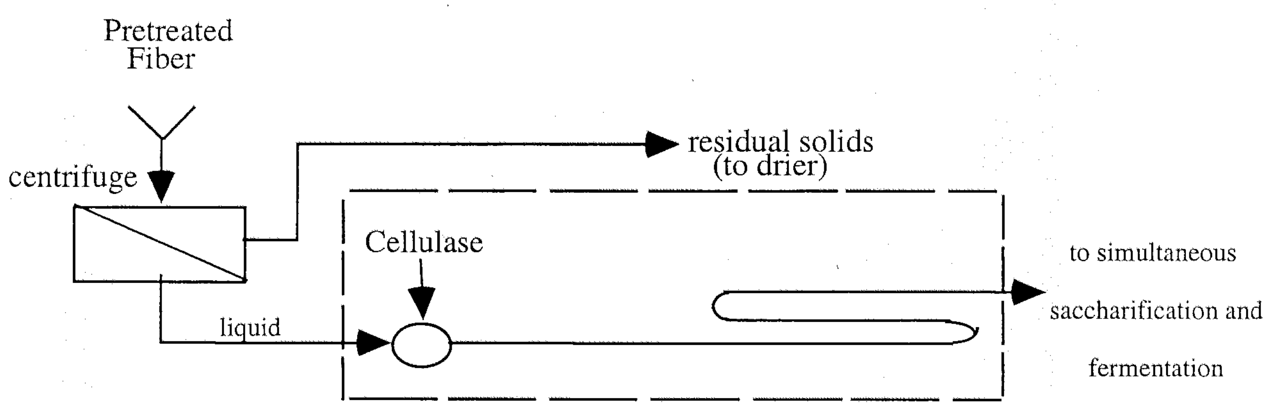
Key elements of the process were tested in the laboratory or pilot plant in order to confirm validity of key design assumptions. These results and the pro-forma results indicate areas for further testing, in preparation of building a pilot scale unit. A key issue will be the material handling properties of the corn fiber system, and the type of equipment that might be needed to handle it. Integration of the equipment in the way that compliments other unit operations and material flows is addressed in the process diagrams. The flow diagram and utility requirements are based on equipment quotations from vendors and Williams operating experience.

Pekin (Williams) Energy has provided engineering expertise, outside design engineering as needed, in order to specify and obtain equipment design and process integration that is realistic and compatible with wet-milling plants. A pro-forma and sensitivity analysis on the effects of capacity on additional capital of operational costs, and the impact of feedstock costs on the production costs of sugars and ethanol are given in detail. The hydrolysis process parameters were based on obtaining enzymes from a commercial source (Genencor) as well as on tests run on pretreated fiber by Genencor in cooperation with our team. Material and energy balance data was based on test runs, confirmed design parameters, and general design guidelines. The conversion process is to be a retrofit, placed at a point in the process where the fiber has been separated from the starch stream. A simplified schematic summarizes the process concepts:

1. Pretreatment:



2. Recovery:



The above diagrams represent the process currently under examination for installation in the Pekin/Williams Energy corn wet-mill. Other possible processes, including the stover conversion process, would send the entire pretreated stream to a separate fermentation tank and a separate beer column, so that the solids could be enzymatically hydrolyzed as well as the liquid.

Special attention was given to the overall water balance, as well as energy balance. We needed to use a pretreatment process that would use as little water as necessary, and the planned process uses no excess water. The water entering the pretreatment process is contained as moisture in both the stillage (7% solids) and fiber processing (37% solids) streams and at the completion of the pretreatment process, the excess water is returned to the plant, or vaporized in a drum dryer. Engineers from Pekin (Williams) Energy participated as partners with Purdue to develop process plans, specify proposed modifications to assure industrial compatibility, and specify key operational parameters for such a process. Further tests under the direction of Pekin and Purdue Engineers and equipment suppliers are recommended in order to finalize process parameters and equipment selection for the pretreatment process.

Fermentation of the pentoses was examined in cooperation with USDA Peoria Laboratories. Their facilities are located within 15 miles of the Pekin plant, hence enabling cooperation in obtaining samples, pretreating materials in tests at the plant, and confirming fermentability. The conditions, times, yields, and requirements for a co-located biomass conversion facility were incorporated into the process flow diagrams, as well as into an analysis of the effects of added capacity, required additional capital, cellulase enzyme costs and handling, and incremental production costs of the resulting sugars. Dr. Bruce Dien of the USDA NCAUR completed experiments to determine if the pretreated corn fiber would cause inhibition of yeast and recombinant bacteria. It was determined that the yeast is unaffected by the pretreated corn fiber, but the hexose and pentose fermenting bacteria were inhibited by the pretreated corn fiber. Parameters for calculating the economics of using cellulase enzymes were obtained from NREL, and from Genencor.

Technical Overview

The crystalline structure of cellulose in corn fiber and corn stover can be made amorphous by pretreatment. Amorphous cellulose is more easily hydrolyzed by cellulases to glucose than crystalline cellulose. The glucose can be used for ethanol fermentations.

The pretreatment process consists of several steps. First, the substrate feed enters a storage tank where the solids (corn fiber or corn stover) and liquid (stillage or water) are mixed. The resulting slurry is pumped through two heat exchangers. The first heat exchanger transfers heat from the fiber/stover stream leaving the pretreatment reactor to the fiber/stover entering the pretreatment reactor. The second heat exchanger transfers heat from steam to the fiber/stover stream in order to bring the slurry to pretreatment temperature. The hot fiber/stover stream enters the pretreatment reactor and is held at 160° or 180°C (for corn fiber and corn stover, respectively) for 30 minutes. It is during this time that the cellulose structure loses its crystallinity. The fiber/stover stream leaves the pretreatment reactor and is cooled by exchanging heat with the incoming fiber/stover stream.

The pretreated fiber/stover stream is then enzymatically hydrolyzed and fermented to ethanol in a simultaneous saccharification fermentation process step. Economic analysis of the conversion by pretreatment of corn fiber to ethanol has been carried out, and using the current technology has been shown to be an economically viable process. The price per gallon of converting corn fiber to ethanol by this method, which would be practical for an operating corn wet-milling ethanol plant, has been calculated to be between $0.77 and $0.87/gallon. The cost of corn stover conversion is much higher if the resale value of solids that remain after pretreatment and hydrolysis is assumed to be zero. If the resale value of remaining solids is equivalent to their wet combustion value, the cost for corn stover conversion is in the range of $1.04 to $1.23/gallon, when the corn stover is processed within the battery limits of a wet-milling plant.

Fermentation of xylose for both the fiber and corn stover cases will reduce ethanol costs. Costs for corn fiber with xylose fermentation will be in the range of $0.74 to 0.83/gallon. Costs for corn stover would be $0.67 to $0.80/gallon. If the arabinose were fermented, in addition to the xylose, ethanol costs would be $0.72 to $0.80/gallon for fiber and $0.64 to 0.77/gallon for corn stover.

The take-home messages from our pro-forma analysis is that:

(1) Corn fiber conversion to ethanol based only on fermentation of glucose hydrolysate approaches an economically interesting range.
(2) Corn fiber conversion based on fermentation of glucose and xylose makes the costs even more attractive.
(3) Co-product credits for corn stover are essential to attain a cost of less than $1.00/gallon.
(4) Fermentation of both glucose and xylose will make corn stover an economically attractive substrate for an existing wet-milling plant.

However, further technical developments are needed.

Next Steps

The analysis and pro-formas resulting from the cooperative efforts of Pekin (Williams Energy Services), Purdue University and USDA NCAUR Laboratory (Peoria) have defined the next steps that must be taken to integrate a corn fiber to ethanol process in the Pekin plant. The purpose of this process would be to test the equipment and operating conditions, and confirm unit yields and unit costs of the fiber pretreatment/hydrolysis/fermentation process at a pilot plant scale. The fiber composition is 36.3% glucans, 16.8% xylan, 10.8% arabinan, 3.5% galactan, 11.8% protein, 8.4% Klason lignin, 2.7% crude fat, 1.8% acetyl group, 0.6% ash, and 7.3% other. This composition was reported in the literature, and is similar to the composition determined in our laboratory. The pro-formas indicate attractive economics. The potential incremental ethanol production increase, based on glucans alone, is an approximately 13% on a total plant basis.

Before such a fiber process would be implemented on a full-plant basis, a pilot plant trial is needed since this is a first-of-a-kind process. The capital cost of the pilot plant scale process is estimated to be in the range of $810,000 to $1.3 million. In this case the key pieces of equipment are two heat exchangers, a centrifuge, pumps, valves, pressure vessels hold tank (i.e., pretreatment reactor, and storage tank), and possibly a mill (grinder). The special properties (specifically viscosity) of the fiber streams will require that the heat exchangers be appropriately specified. This will require tests at the manufacturer (Alfa-Laval) or the Pekin plant. Similarly, the dewatering efficiency of the centrifuge will need to be confirmed at a realistic scale at the manufacturer's site (Sharples-Alfa-Laval). Tests of the pumps will be done at Pekin. Intermediate (50 lb. lots) and large (200 lbs.) volumes of both untreated and pretreated corn fiber will need to be generated and analyzed for these tests. The smaller lots of material will be prepared at Purdue, while larger scale lots will be processed at Pekin. While a mill (grinder) has not been necessary for corn fiber, this contingency must be considered if plugging of the heat exchanger is found to occur, when the manufacturer's trials are carried out.

As larger scale trials are carried out, a significant analytical workload will also result since changes in fiber composition and hydrolysis characteristics will be monitored as the steps are scaled up. These characteristics will include enzyme hydrolysis, confirmation of absence of fermentation inhibitors, flow characteristics, and ethanol yields at simulated process conditions. Enzyme treatment levels will be examined on a larger scale, in order to further confirm and tighten cost ranges. Runs for corn stover will be carried out at the same time, in order to obtain further data on it, although the focus of the first trial will be for corn fiber.

Some of the cost parameters which will be further tightened as a result of manufacturer trials include robustness of the equipment, electrical cost for pumping and centrifugation, energy costs of pretreatment as a function of heat exchanger efficiency, cooling water requirements, and labor, facility, and miscellaneous reagent costs. While there are expected to make only small contributions to the cost of ethanol production, they must still be defined.

Information on the process financing and rates of return on the process is currently unavailable.

Other considerations which will be important in fielding the first corn fiber plant in an existing facility include:

> The interaction of the fiber pretreatment process and the manufacture of these other products and the economic impact of the pretreatment process on sales of secondary products from the ethanol plant.
>
> It was assumed in the flowsheets that the pretreated corn fiber residual solids could be fed to stock animals and that it would have the same sales value. These assumptions need to be verified.
>
> Longer term, the microorganism used for fermentation of the pentoses will need to be selected. Currently, there are a limited number of microorganisms that can ferment both hexoses and pentoses to ethanol, and they will need to be evaluated.
>
> The corn fiber process must be designated so that it adds value without negative effects on existing co-products. These would include fiber, feed, gluten, corn oil/germ, and other co-products.

Another important and related area is optimization of enzyme levels/fermentation conditions. There will be energy savings for the plant from having less corn fiber to dry at the final fiber-drying step, due to hydrolysis and solubilization of the fiber. This will be offset by the additional energy needed to run the solid bowl centrifuge, pumps, and agitator motors for the storage and fermentation (only in separate fermentation processes) tanks, which are additional pieces of capital equipment used in the pretreatment process. The design factors for the solid bowl centrifuge are currently being determined by Alpha Laval from a sample of pretreated fiber and stillage. When these results are determined, the costs and savings associated with fiber drying in the plant will be known. The energy needed to run the solid bowl centrifuge will be determined once the specifications for the centrifuge are determined.

Additional costs also need to be determined: labor costs, facility electricity and reagent costs. Labor costs and maintenance and sampling costs will need to be added, as well as lab costs for the samples taken from the pretreatment process line. Facility electricity costs will need to be determined, including electricity for the pump and stirring motors and other electricity uses. Reagents used in cleaning out the fermentation tanks and lines and other uses of reagents in the process line will need to be determined.

The current enzyme treatment level used in the flowsheets was suggested and reconfirmed by Genencor. They are currently determining the optimum mixture of enzymes and the optimum level of enzymes needed for hydrolysis.

The specifications for the heat exchanger were determined by Alpha Laval, based on data given to them by this lab. This heat exchanger design will have to be tested to determine if the fiber and/or stover streams will be too viscous, or if solids concentration is too high for the width of the passes. If so, a fiber grinder may be necessary, or perhaps a redesign of the heat exchangers. Also, the pumps will need to be tested to determine if they will pump the pretreated slurry at the needed flow rates.

Objectives

The objectives of this contract were threefold. First, a process was designed, based on experimental knowledge and industrial experience, to incorporate a corn fiber pretreatment/ enzyme hydrolysis/ ethanol fermentation system into an existing corn starch-fermenting ethanol plant. The second objective was to incorporate a corn residue pretreatment, enzyme hydrolysis, and ethanol fermentation system into an existing corn starch-fermenting ethanol plant. The final objective was to carry out an economic analysis of the key process steps and generate a pro-forma analysis for corn fiber and corn stover pretreatment/ enzyme hydrolysis/ ethanol fermentation.

Approach

Corn Fiber

We experimentally confirmed the key chemical compositions of corn fiber by enzyme hydrolysis combined with HPLC. The method used is outlined in Saha and Bothast (1999) with adjustments. Seven grams of dried (at 70°C), ground (to 40 mesh) corn fiber was slurried in 50 mL of 0.5% sulfuric acid. 3.3 mL of 10M sodium hydroxide solution was added to reduce the pH from 1.6 to 5.5. 5 mL of Spezyme CP was added to the fiber at 50°C and the total hydrolysis time was 155 hours. The resulting material was filtered on a Whatman cellulose filter and the resulting liquid was analyzed by HPLC analysis on a Bio-Rad HPX-87H ion-exchange column operated at 60°C. The starch percentage was determined by taking 7 grams of ground corn fiber and adding 50 mL of pH 7.0 Tris(hydroxymethyl)amino-methane buffer at 37°C. 5 mL of α-amylase and 6 mg of amyloglucosidase were added and the total hydrolysis time was 155 hours. The mixture was filtered on a Whatman filter and the liquid was analyzed by HPLC analysis. These two analyses gave the total xylose, arabinose, galactose, and glucose percentages as well as acetic acid and protein percentages. The ash percentage was determined by ashing the fiber at 600°C in a muffle oven. The lignin was analyzed by NREL Chemical Analysis & Testing Standard Procedures 003: Determination of Klason Lignin in Biomass and 004: Determination of Acid Soluble Lignin in Biomass.

Genencor, who has worked with Williams Energy and this lab, completed experiments concerned with the extent of hydrolysis, and both Genencor and Dr. Bruce Dien at USDA studied the fermentation characteristics. We obtained realistic cost ranges for steam energy, fiber, enzyme, and capital from industrial and printed sources.

Bruce Dien ran shake flask fermentation experiments on the pretreated corn fiber with yeast (from Alltech) and two *E. coli* strains K011 and FBR5 (developed at the USDA Peoria lab) to determine if the pretreated corn fiber would inhibit growth of ethanol producing microorganisms. The yeast experiments were conducted using the actual fermentation medium from Pekin/Williams Energy consisting of light corn steep liquor and well water. In the experiments, the pretreated corn fiber, light corn steep liquor and well water were mixed in ratios of 0:1:1, 1:1:2, 1:0:1, 1:2:1, and 1:1:0. The yeast was added at a level of $2x10^7$ cells/ml. The *E. coli* experiments were conducted using a Luria broth supplemented with 5% w/v xylose. The medium was inoculated with *E. coli* at an OD (550 nm) of 0.1. See the Appendix for the details of the experiments.

The results of the substituted pretreated corn fiber "appears to have no significant effect when used to replace either the corn steep liquor or well water" (Dien personal communication, 1999) on the growth of the yeast. These results are for pretreated corn fiber that has not been hydrolyzed, so the monomer concentration is low. The time course fermentation graphs for the yeast fermentations are found in Figure 1 and Figure 2. The weight loss in the Y-axis is correlated to the amount of carbon dioxide produced during the fermentation. As can be seen, the weight losses during all the fermentations are very similar and no definite pattern can be related between the six fermentation conditions.

The *E. coli* experiments, however, show definite inhibition for both the *E. coli* K011 and FBR5 strains in the presence of pretreated corn fiber, as shown in Figure 3. Even when the initial inoculum was grown in the presence of pretreated corn fiber (Figure 4) the ethanol yield is not improved for fermentations including the pretreated corn fiber. Finally, it was seen that even the growth of the inoculation is hindered by the presence of pretreated corn fiber (Figure 5). These results indicate that removal of fermentation inhibitors may be needed if a genetically modified bacterium is to be used as the fermentation organism.

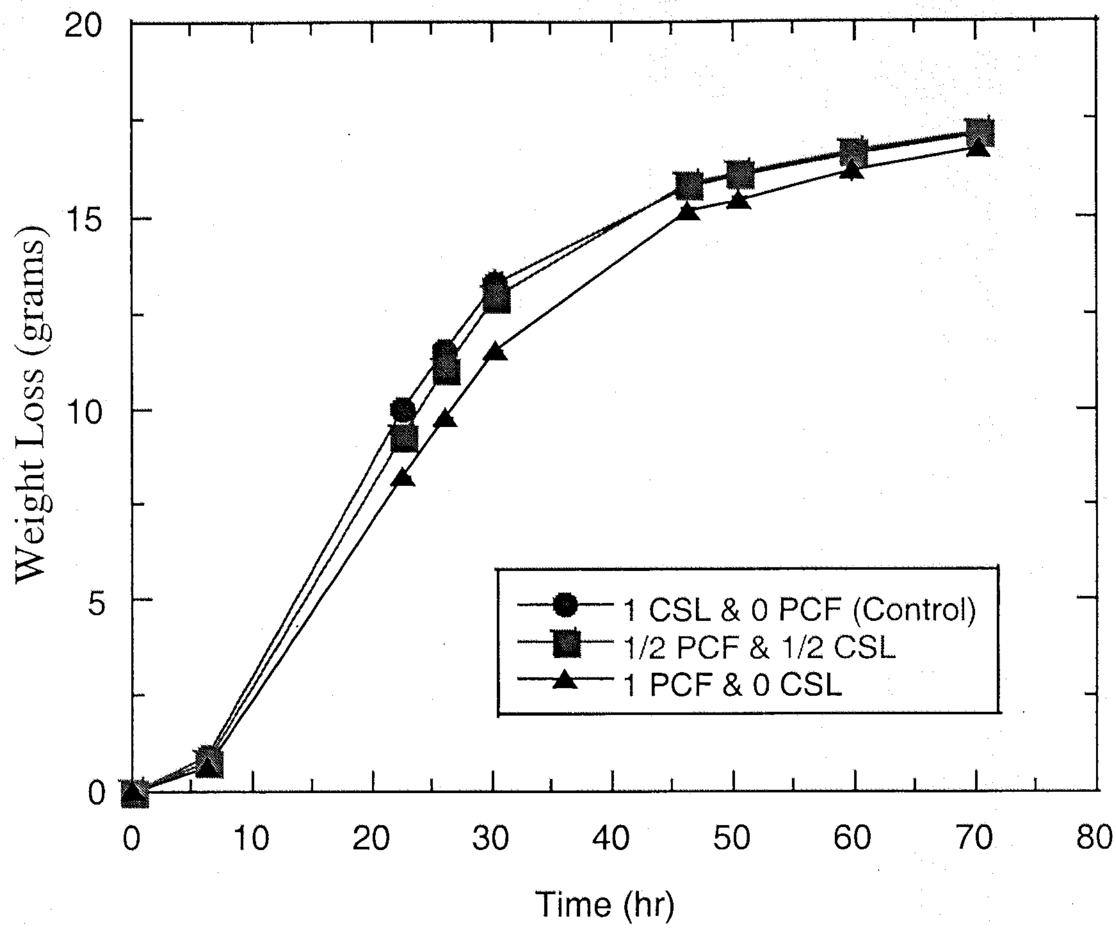


Figure 1: Time Course Fermentation Detailing the Effect of Substituting Pretreated Corn Fiber (PCF) for Corn Steep Liquor (CSL). The control is based on the actual fermentation medium used in the Pekin/Williams Energy plant. The PCF is not substituted, substituted for half of the CSL, or completely substituted for the CSL.

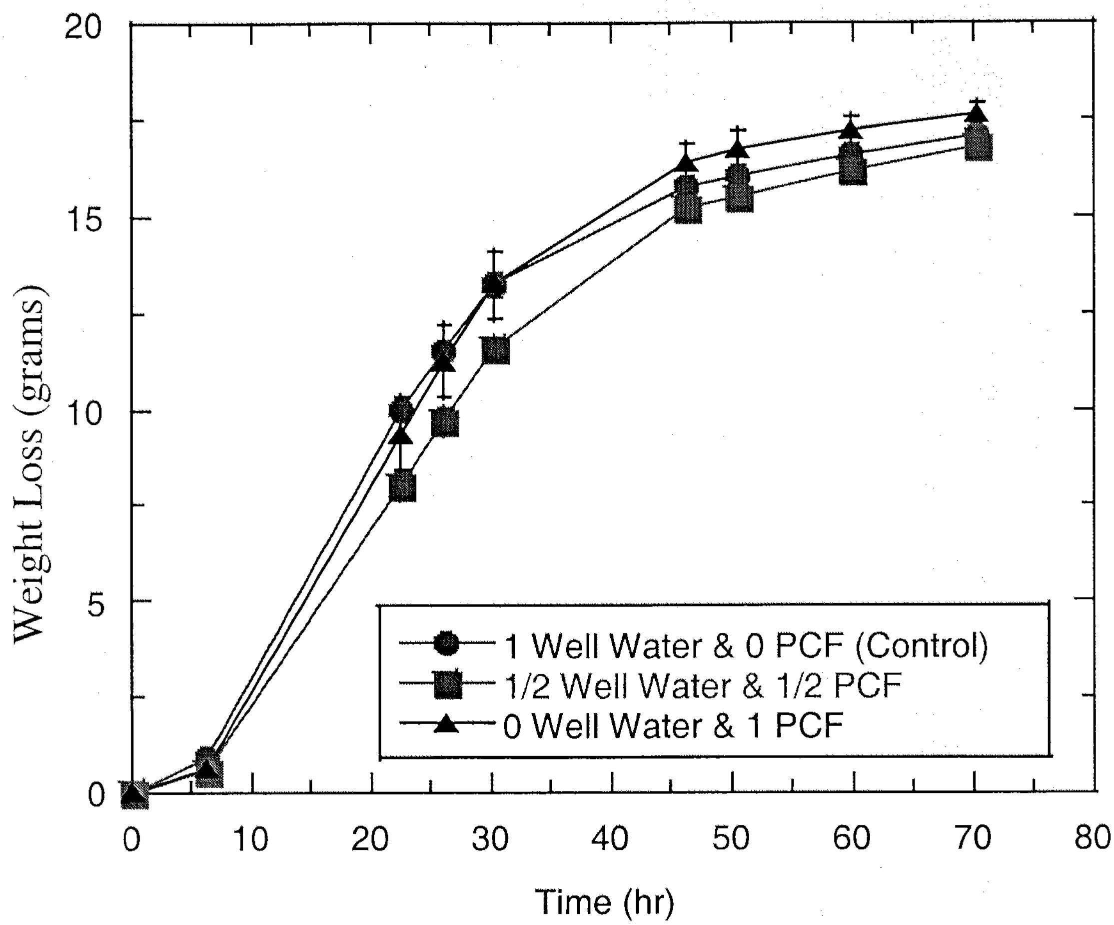


Figure 2: Time Course Fermentation Graph Detailing the Effect of Substituting Pretreated Corn Fiber (PCF) for Well Water. The PCF is not substituted, substituted for half of the Well Water, or completely substituted for the Well Water

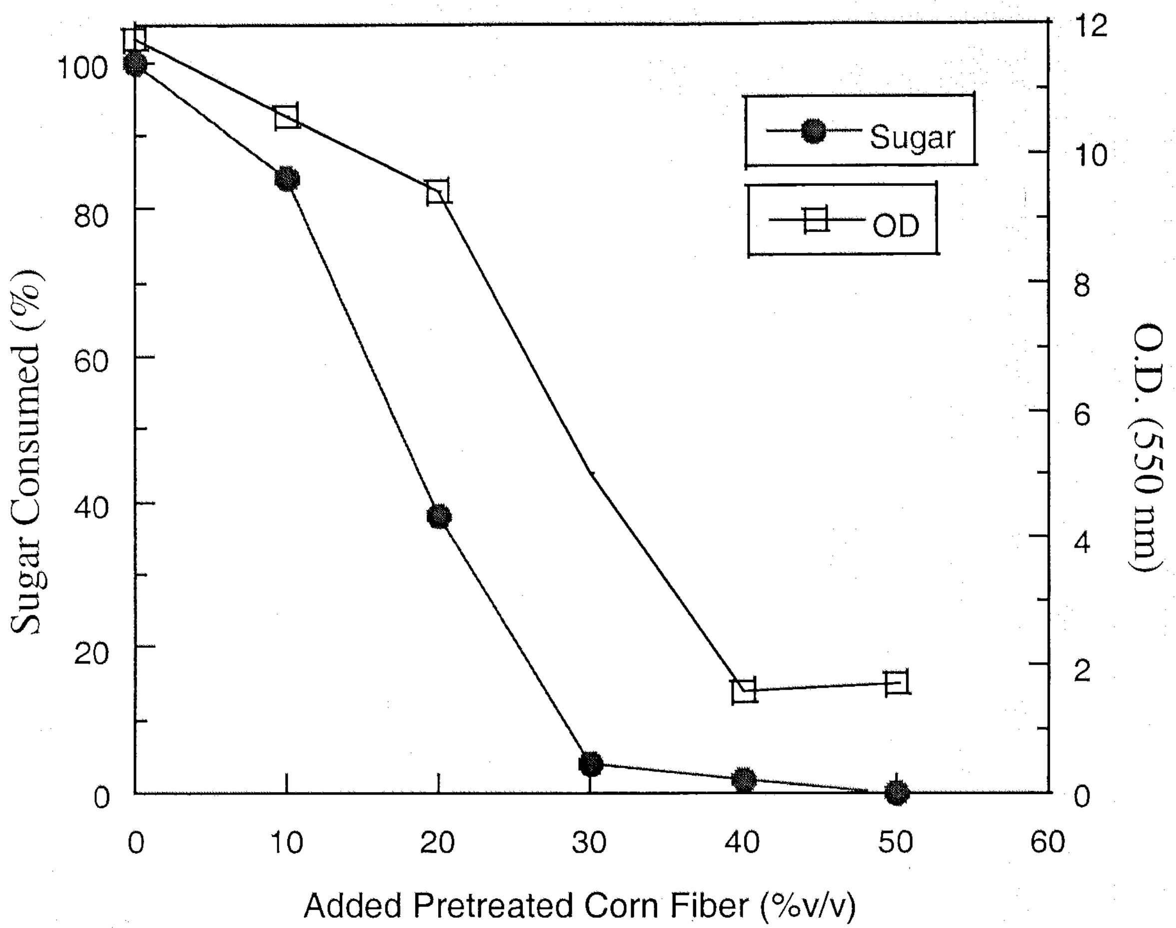


Figure 3: Growth of E. coli K011 Seed Cultures on Medium Containing Varying Concentrations of Pretreated Corn Fiber. The fermentation was completed at 30°C, pH 6.5, inoculated to 0.1 OD. The data points are after 24 hr. The ethanol and sugar analyses were completed by HPLC. The fermentation medium was LB medium supplemented with 5% w/v xylose.

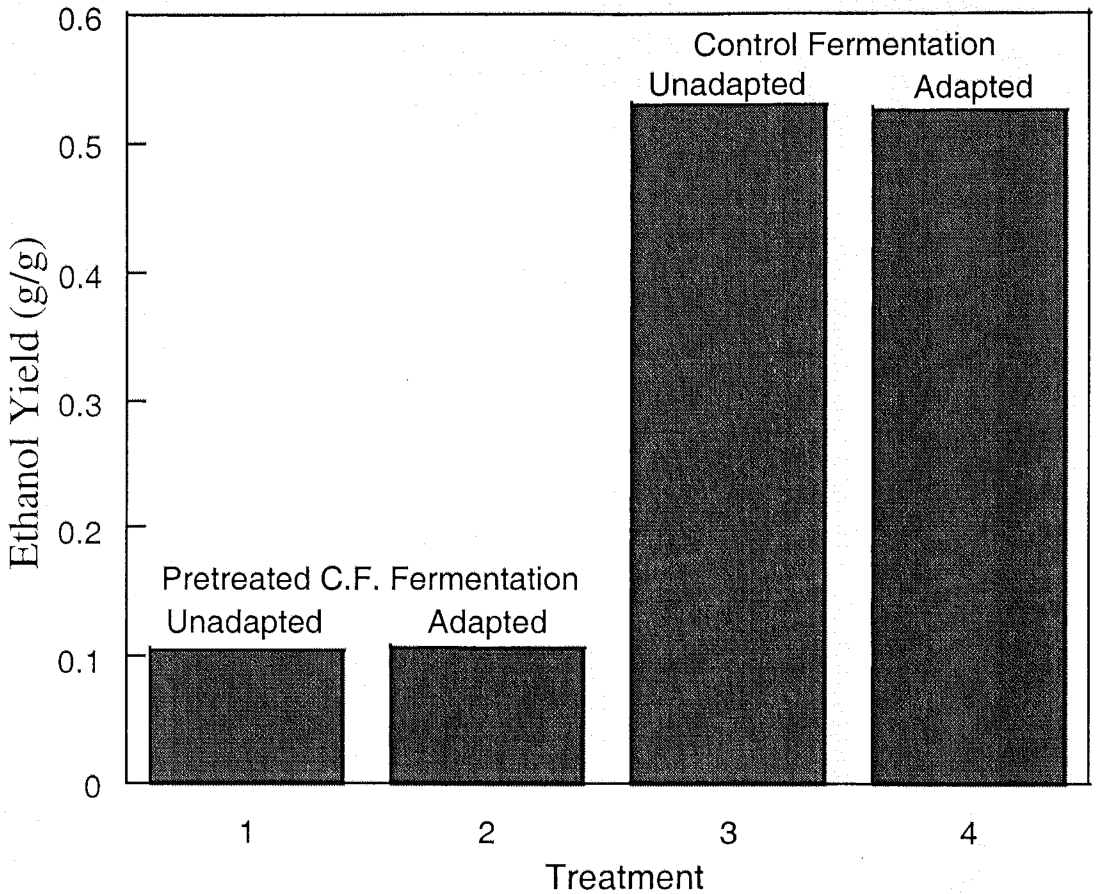


Figure 4: Effect of growing *E. coli* K011 seed culture in presence of Pretreated Corn Fiber (PCF) on subsequent fermentation. This fermentation was completed at 35°C, pH = 7; in tryptone and yeast extract and either a 0.1 M Pipes buffer or mixture of sugars control. The fermentation was checked every 24 hours. The ethanol yield was determined by HPLC.

Table 1: Description of Treatments for Figure 4

Treatment	Seed Culture	Fermentation
1	Not adapted	PCF
2	Adapted to PCF	PCF
3	Not adapted	Mixed sugars
4	Adapted to PCF	Mixed sugars

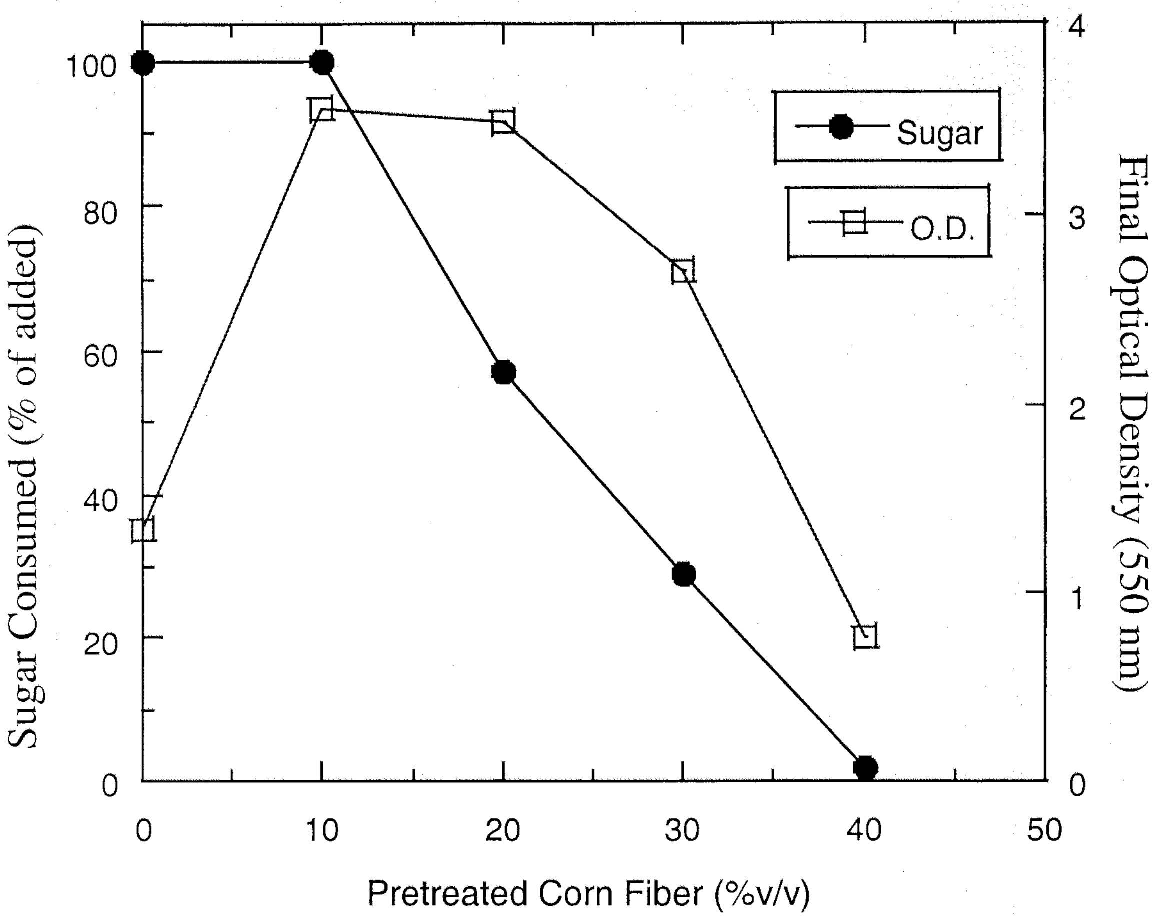


Figure 5: Effect of Adding Pretreated Corn Fiber on the Seed Culture of *E. coli* FBR5. The fermentation was completed at 37°C for 12 hr. The fermentation medium was anaerobic LB medium supplemented with 4 g/l xylose.

Corn Residue

The extent of experimentation was sufficient to determine handling properties, compositions, and hydrolysis characteristics. The methods used to determine the composition of corn stover were NREL Chemical Analysis & Testing Standard Procedures 001: Determination of Total Solids/Moisture in Biomass, 002: Two Stage Sulfuric Acid Hydrolysis for Determination of Carbohydrates, 003: Determination of Klason Lignin in Biomass, 004: Determination of Acid Soluble Lignin in Biomass, and 005: Standard Method for Ash in Biomass. The compositions and hydrolysis characteristics were determined during experimental runs completed in this lab.

Compositions

Table 2: Compositions of Stillage and Corn Fiber (literature and experimental)(dry basis)

	Stillage	Corn Fiber*	Corn Fiber Experimental⁺
DP4	9.2%		
DP3	2.4%		
DP2	14.8%		
Glucose	1.2%		
Xylose	2.1%		
Succinic Acid	1.5%		
Lactic Acid	14.2%		
Glycerol	20.6%		
2,3 Butanediol	6.4%		
Ethanol	1.6%		
Protein (BCA)	0.8%		
Crude Fat		2.7%	2.5%
Klason Lignin		8.4%	7.8%
Ash		0.6%	0.4%
Acetyl Group		1.8%	2.0%
Solids			
Glucan	6.5%	36.3%	35.2%
Xylan	0.3%	16.8%	12.3%
Galactan	0.1%	3.5%	3.2%
Arabinan		10.8%	11.3%
Soluble Fiber			6.5%
Protein	11.8%	11.8%	
Protein + DNA			10.2%
Ash (Insoluble)	0.4%		
Other (by weight)	6.1%	7.3%	8.5%
Total	100.0%	100.0%	100.0%

*Saha, B.C. and R.J. Bothast "Pretreatment and Enzymatic Saccharification of Corn Fiber" Applied Biochemistry and Biotechnology, **76**, 65-77. (1999)

⁺Experimental refers to procedures done in the Purdue University laboratory.

Unit Costs and Values

Unit costs and values change rapidly, but the current values and ranges are shown in Table 4. Fluctuations in these commodities and utilities can have a large effect on the cost of producing ethanol, as will be shown in the later graphs and tables. As can be seen, the current values of corn fiber and corn stover are low and the value of ethanol is high, and this combination is favorable for biomass conversion.

Table 3: Composition (literature and experimental) of Corn Stover

	Corn Stover Experimental+	Corn Stover^
Klason Lignin	21.5%	
Protein		3.9%
Glucan	38.0%	39.0%
Xylan	26.3%	20.1%
Arabinan	3.4%	2.0%
Lignin	2.8%	21.5%
Ash (Insoluble)	8.0%	6.8%
Other		6.7%
Total	100.0%	100.0%

^Kaar, W.E. and M.T. Holtzapple "Benefits from Tween During Enzymic Hydrolysis of Corn Stover" Biotechnology and Bioengineering, **59** (4), 419-427. (1998)

+Experimental refers to procedures done in the Purdue University laboratory.

Table 4: Ranges and Current Cost/Value of Several Commodities and Utilities

	Range	Current Cost/Value
COSTS		
Energy Costs ($/Million BTU)	1.80-5.00	2.50
Enzyme Costs ($/kg enzyme)	5-6	5-6
Stover Costs ($/dry ton)	35	35
VALUES		
Fiber Value ($/dry ton)	35-120	65
Ethanol Value ($/gallon)	0.90-1.30	1.00

Results

Corn Fiber

Corn Fiber-Liquid Fermentation Only

This case is specialized for wet-milling ethanol plants where only liquid can enter the corn starch fermenters. In this particular case, after the fiber and stillage are pretreated at 160°C, the fiber enters a solid bowl centrifuge. The flowsheet for this case is shown in Figure 6. In this process, whole, unground corn enters the plant and is cleaned and stored. The corn is then sent to a steeping tank where it is allowed to rehydrate. The corn is then sent to degermination milling and germ separation, where the germ of the corn is removed and processed. The pericarp of the corn is then removed from the endosperm and processed in the fiber processing line. The starch and gluten are then separated with the gluten going to the gluten processing line and the starch going to starch storage. The stored starch is hydrolyzed to glucose with amyloglucosidase and α-amylase. The glucose goes into a fermenter to be fermented to ethanol.

The fiber from the fiber drying line is sent to the pretreatment process, which is detailed in Figure 7. In this pretreatment process, the fiber and stillage are first pumped and mixed in a storage tank. The combined fiber/stillage stream is then pumped into two heat exchangers. The first heat exchanger transfers heat from the fiber leaving the pretreatment reactor to the fiber leaving the storage tank. The fiber then goes through a second heat exchanger that transfers heat from steam to the fiber stream. The fiber stream is then held at 160°C for 30 minutes in a pretreatment reactor, and finally passes through the first heat exchanger as the heat source. The stream is then separated into a solids stream and a liquid stream by a solid-bowl centrifuge. The liquid is sent to the starch fermenters and the solid is sent to the fiber drying line. The amount of liquid entering the starch fermenters is based on the amount of fiber that solubilizes experimentally during pretreatment. The liquid is hydrolyzed in the fermenter and fermented to ethanol along with the glucose from the starch. The ethanol stream is sent to a distillation system where the ethanol leaves as a vapor and the water leaves as the bottoms.

Figure 8 and Figure 9, respectively, show the total and incremental ethanol produced from the fiber when specific sugars are fermented on a 1000 lb_m/day plant inlet basis. The starch line, in this 1000 lb_m/day plant inlet basis, would produce 51 gallons of ethanol/day. Figure 10, Figure 11, and Figure 12 show the cost of ethanol per gallon based on fiber, enzyme, capital equipment, and energy costs for the liquid-only stream fermentation.

As can be seen from Figure 9, the largest contribution of incremental ethanol is the DPs. DP stands for "degree of polymerization", so DP2, DP3 and DP4 would simply be chains of 2, 3, and 4 monomers, respectively. In the case of corn fiber hydrolysis, the DPs will be multiple pentose and dextrose units. The analysis of the pretreated corn fiber by HPLC cannot separate the DP molecules that are dextrose polymers from those that are xylose or arabinose polymers. By enzymatically hydrolyzing these DPs with cellulases, xylanases and arabinases, monomeric sugars will be formed, which can be used for ethanol fermentation. These polysaccharides are only available for ethanol fermentation if the liquid is enzymatically hydrolyzed.

Table 5 shows the detailed costs for the liquid-only fermentation flowsheet. The column headings indicate what sugars will be fermented. The 95% hydrolysis is based on dissolved oligosaccharides in the liquid stream, since only the liquid portion of the pretreated corn fiber stream enters the fermenters. All fermentable sugars means that every pentose and hexose sugar will be fermented to ethanol and the remaining columns are based on the sugars listed. On the last two columns, the DPs are not fermented, which would be true only if enzymatic hydrolysis does not occur. So for those columns the enzyme cost is zero, because it assumes no enzymatic hydrolysis. The column that is of the most interest currently is the third column, Only Glucose, Galactan, and DPs Ferment, because the microorganism that will most likely be used initially, is a simple glucose-fermenting yeast. The costs for reagent use, labor and facility are not able to be accurately determined at this time, but are expected to be the least significant costs.

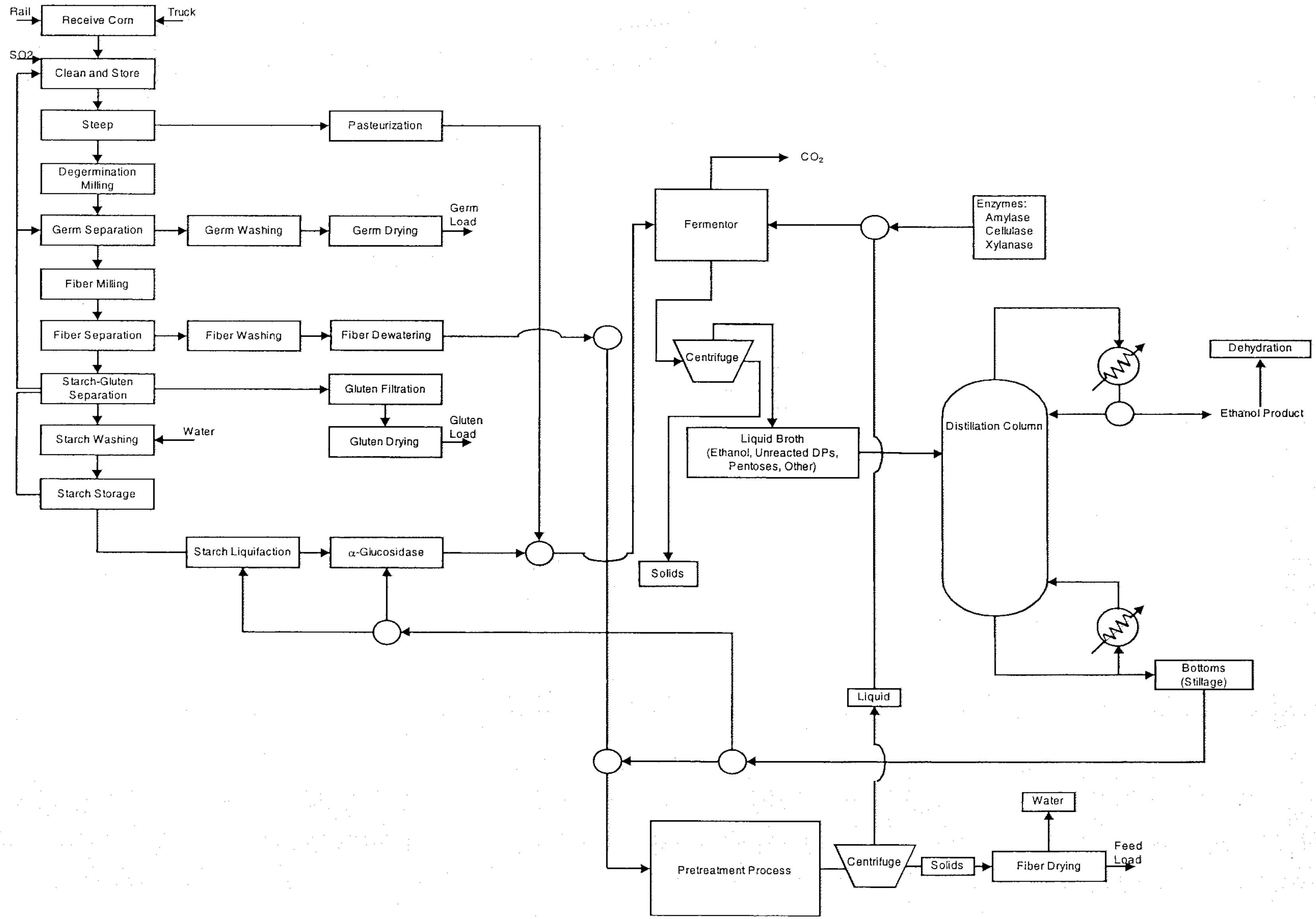
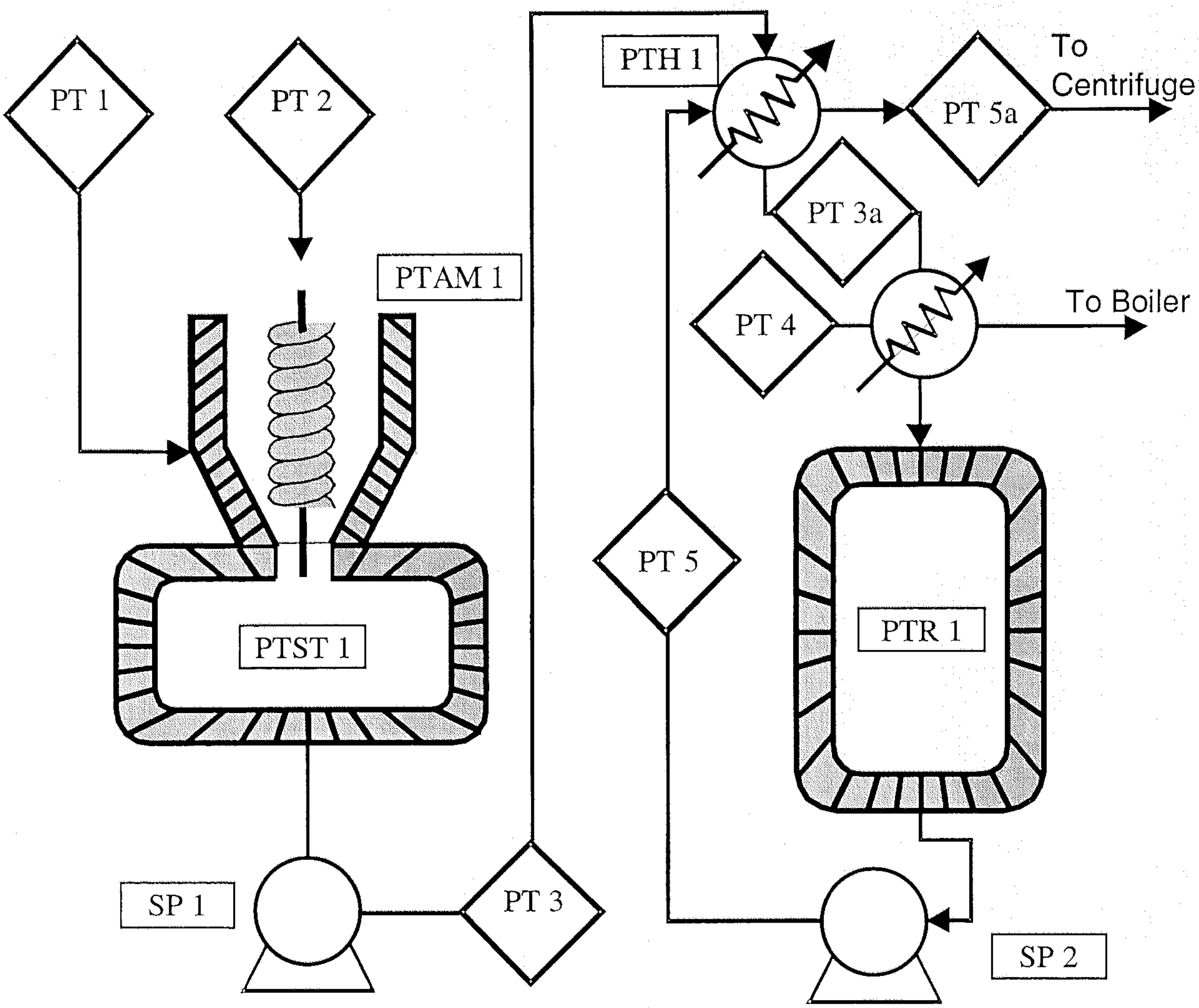


Figure 6: Flowsheet for Corn Fiber Liquid Fermentation Only



PTAM 1 = Pretreatment Auger-Mixer
PTST 1 = Pretreatment Storage Tank 1
SP 1 = Slurry Pump 1
SP 2 = Slurry Pump 2
PTH 1 = Pretreatment Heat Exchanger 1
PTR 1 = Pretreatment Reactor 1

PT 1 = Stillage
PT 2 = Corn Fiber
PT 3 = Pretreatment Heat Exchanger Cold-Side Inlet
PT 3a = Pretreatment Reactor Inlet
PT 4 = Steam
PT 5 = Pretreatment Reactor Outlet
PT 5a = Pretreatment Reactor Heat Exchanger Hot-Side Outlet

Figure 7: Pretreatment Process

The total costs per gallon of ethanol are based on the items in the table. Depending on the cost of energy and the actual installed cost of the new capital equipment, the price per gallon of ethanol will be between \$0.77 and \$0.87 for the conditions of the third column. The major cost for all of the corn fiber and corn stover spreadsheets is the substrate cost, due to the large amount of fiber needed per gallon of ethanol. However, we assume that the residual corn fiber would have the same value as the untreated fiber, so the fiber remaining after hydrolysis could be sold at the same price as non-pretreated fiber.

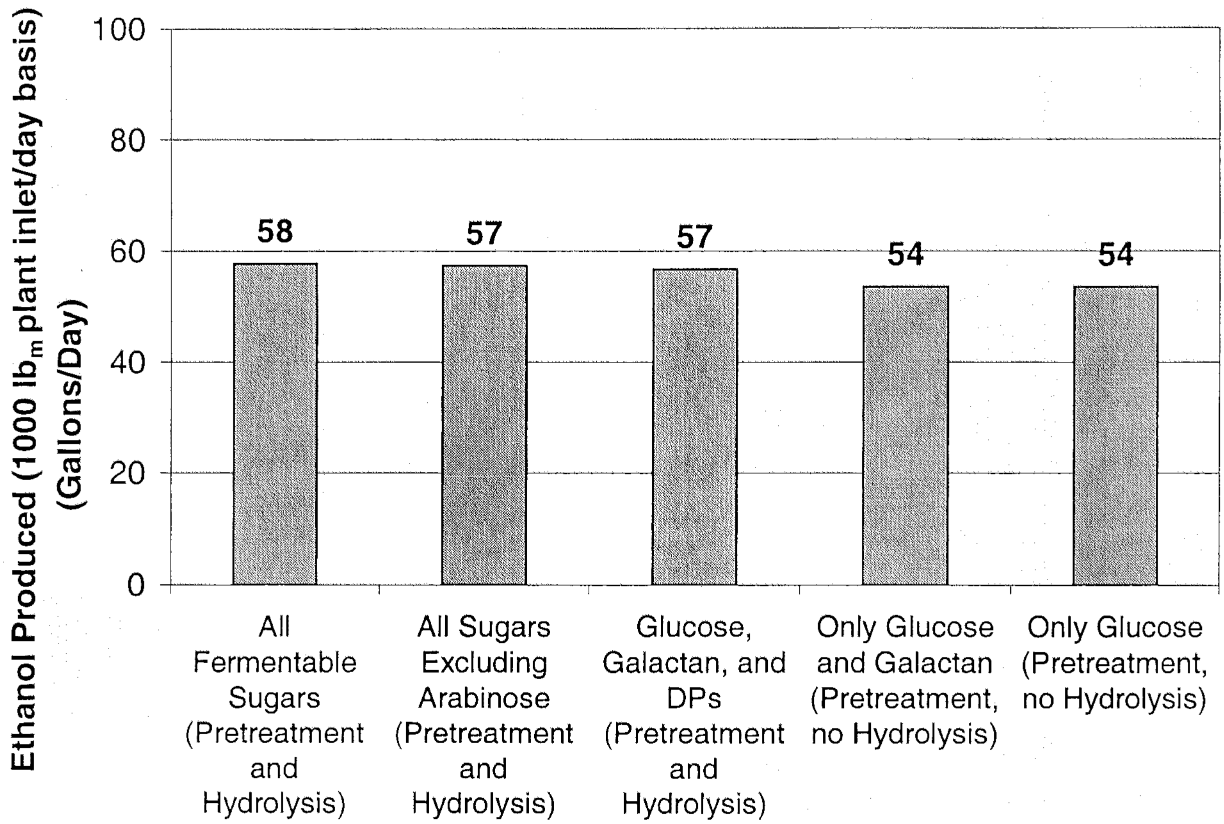


Figure 8: Total Ethanol Produced from Fiber Stream and Starch Stream on a 1000 lb$_m$/day plant inlet basis (where the starch line would produce 51 gallons of ethanol/day) (95% hydrolysis and 90% Fermentation Efficiency)

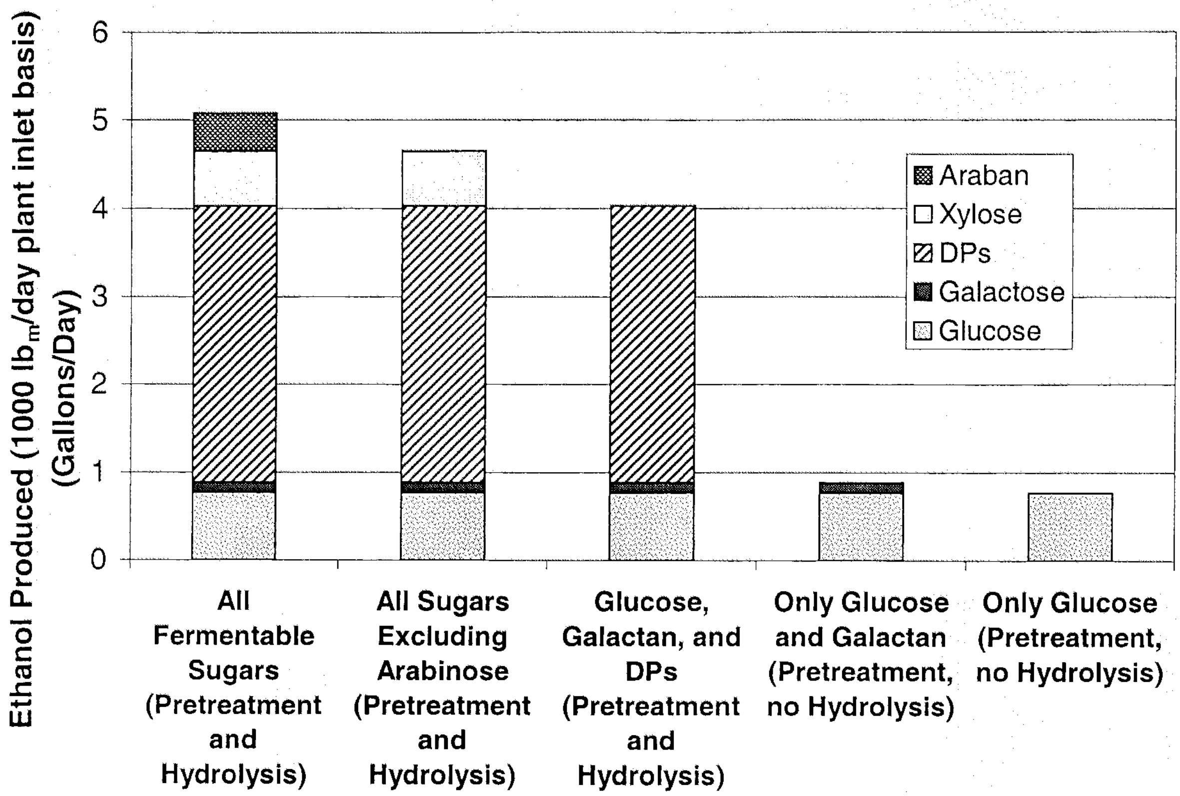


Figure 9: Incremental Ethanol Produced from Only the Fiber Stream on a 1000 lb$_m$/day plant inlet basis (95% hydrolysis and 90% Fermentation Efficiency)

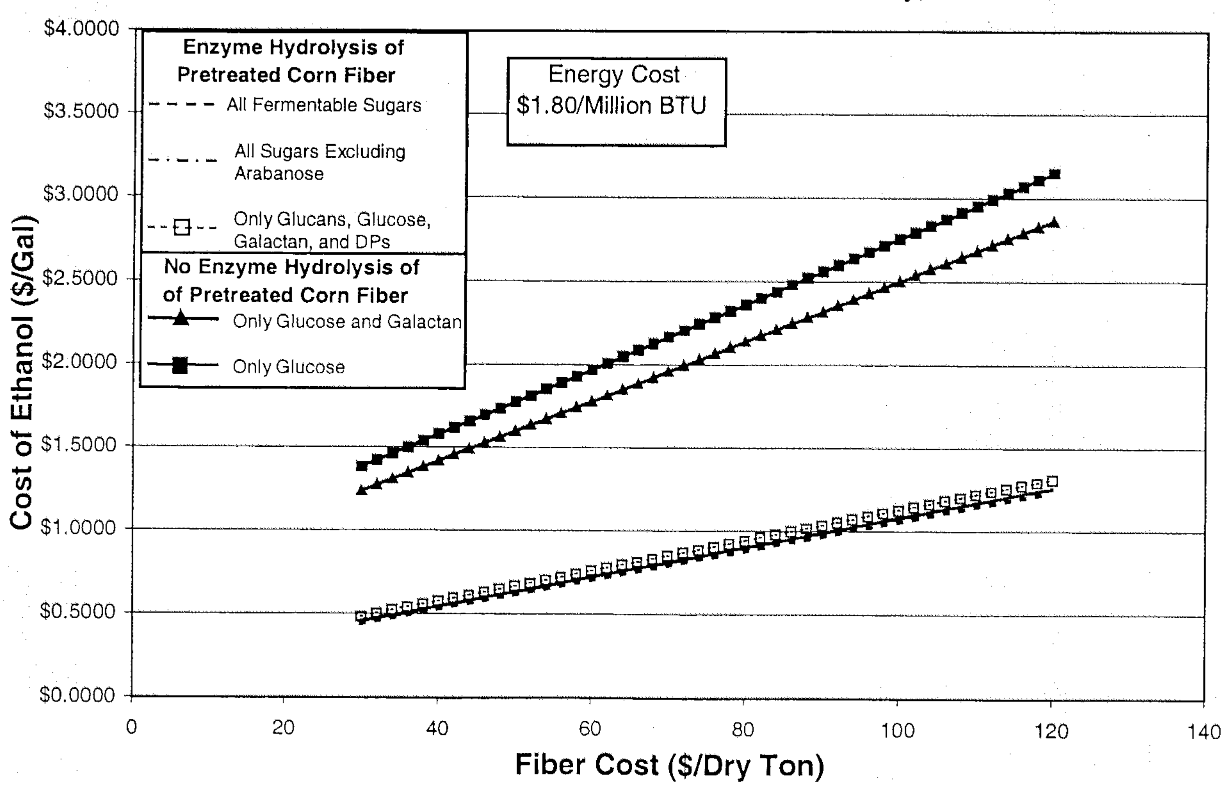


Figure 10: Economics of Ethanol Production for an Energy Cost of $1.80/Million BTU

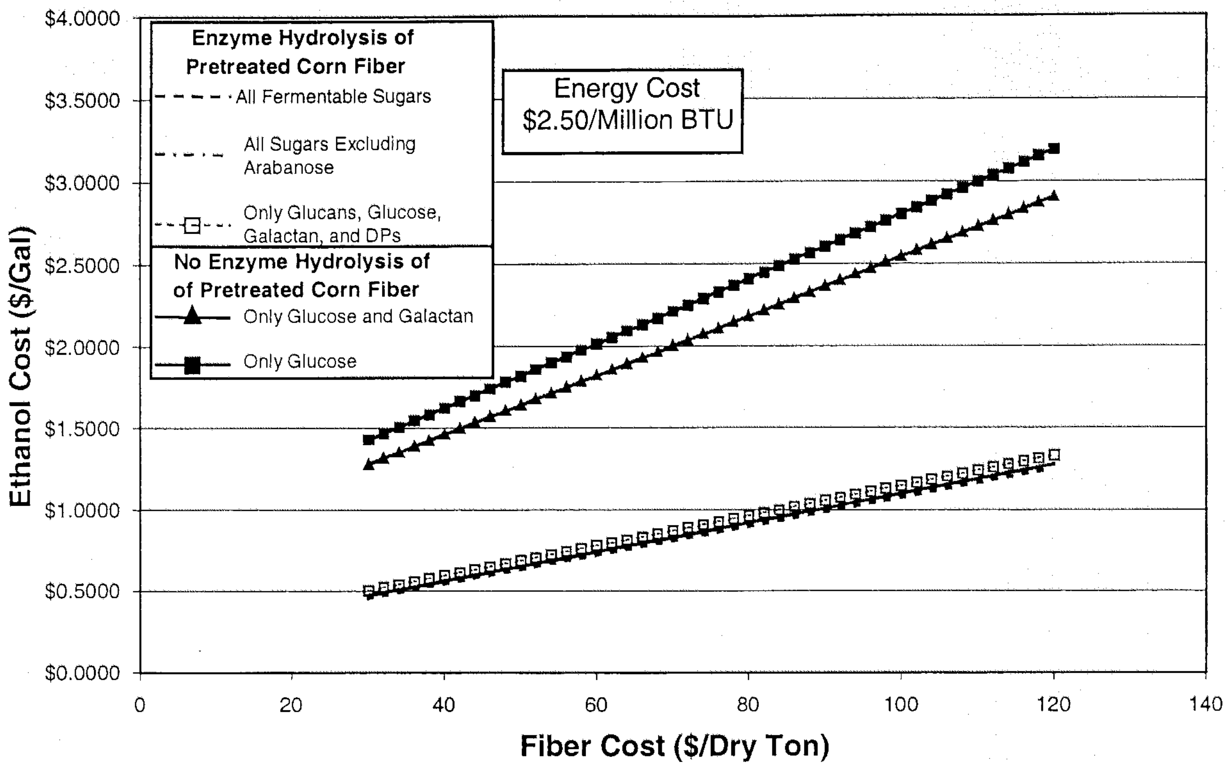


Figure 11: Economics of Ethanol Production for an Energy Cost of $2.50/Million BTU

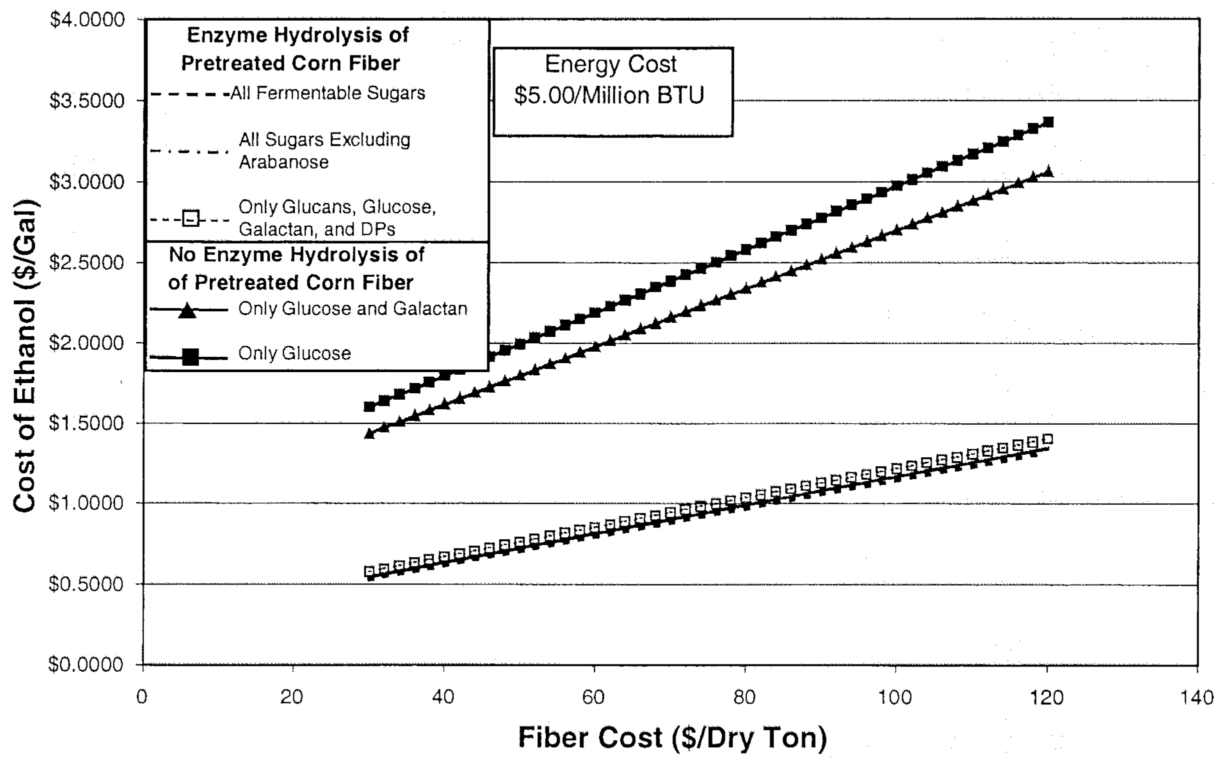


Figure 12: Economics of Ethanol Production for an Energy Cost of $5.00/Million BTU

Table 5: Detailed List of Costs for Corn Fiber Liquid Stream Only (95% hydrolysis of Theoretical and 90% Fermentation Efficiency)

FIBER VALUE ($65) Ethanol Cost ($/gallon)	All Fermentable Sugars	All Sugars Excluding Arabinose Ferment	Only Glucose, Galactan, and DPs Ferment	Only Glucose, and Galactan Ferment	Only Glucose Ferments
Energy Cost for Pretreatment					
@ $5/MMBTU	$0.0372	$0.0405	$0.0468	$0.2121	$0.2435
@ $2.5/MMBTU	$0.0186	$0.0203	$0.0234	$0.1060	$0.1217
@ $1.8/MMBTU	$0.0134	$0.0146	$0.0168	$0.0764	$0.0877
Capital Costs - New Plant	$0.1023	$0.1116	$0.1288	$0.5839	$0.6703
- Retrofit	$0.0840	$0.0916	$0.1058	$0.4793	$0.5503
Energy Cost for Distillation					
@ $5/MMBTU	$0.0705	$0.0705	$0.0705	$0.0705	$0.0705
@ $2.5/MMBTU	$0.0353	$0.0353	$0.0353	$0.0353	$0.0353
@ $1.8/MMBTU	$0.0254	$0.0254	$0.0254	$0.0254	$0.0254
Energy Saved From Evaporating Water From Fiber					
Net Fiber Cost	$0.5746	$0.5824	$0.5995	$1.1737	$1.2747
Enzyme costs	$0.0195	$0.0212	$0.0245	$0.0000	$0.0000
Reagent costs (chemicals)					
Labor costs					
Electricity costs					
Total Cost per Gallon with Steam Costs @ $5/MMBTU					
-New Plant Capital Costs	$0.8040	$0.8263	$0.8701	$2.0403	$2.2590
-Retrofit Capital Costs	$0.7857	$0.8063	$0.8470	$1.9357	$2.1389
@ $2.5/MMBTU					
-New Plant Capital Costs	$0.7502	$0.7708	$0.8115	$1.8990	$2.1020
-Retrofit Capital Costs	$0.7319	$0.7508	$0.7884	$1.7944	$1.9820
@ $1.8/MMBTU					
-New Plant Capital Costs	$0.7351	$0.7552	$0.7950	$1.8594	$2.0580
-Retrofit Capital Costs	$0.7168	$0.7352	$0.7720	$1.7548	$1.9380

Assumptions:
Yearly Value of the fiber after being pretreated is based on fat, protein, and ash in the fiber and starch lines.

Corn Fiber-Entire Pretreated Stream into Starch Fermenter

This case is designed for a plant in which the entire pretreated stream enters the starch fermenters. The amount of incremental ethanol generated from fiber and stillage in this method is shown in Table 6. The flowsheet for this case is shown in Figure 13. This flowsheet is the same as Figure 6, except that the centrifuge to separate the solids from the liquid stream is found after the distillation column.

Table 6 shows that the amount of ethanol produced is greater than that of the liquid stream fermentation-only case on a 1000 lb_m/day plant inlet basis (where the starch line would produce 51 gallons of ethanol/day). Table 7 shows the detailed costs per-gallon of ethanol. The 95% enzyme hydrolysis of the polysaccharides to monosaccharides is a "best case" value. The costs are about $0.10 per gallon less than for the liquid-only fermentation, because all of the pretreatment costs are decreased by the larger volume of ethanol produced. The last two columns contain enzyme costs, because the solid glucans would need to be hydrolyzed, whereas before, there were no solid glucans in the liquid-only stream.

Table 6: Incremental Ethanol from the Fiber Line for the Corn Fiber Entire Stream Case on a 1000 lb_m/day plant inlet basis (where the starch line would produce 51 gallons of ethanol/day) (95% hydrolysis and 90% Fermentation Efficiency)

		All Fermentable Sugars	All Sugars Excluding Arabinose	Glucans, Glucose, Galactan, and DPs	Only Glucans, Glucose, and Galactan	Only Glucans and Glucose
Total Conversion Theoretical Basis	gallons/day	7.74	6.80	5.26	4.21	3.91

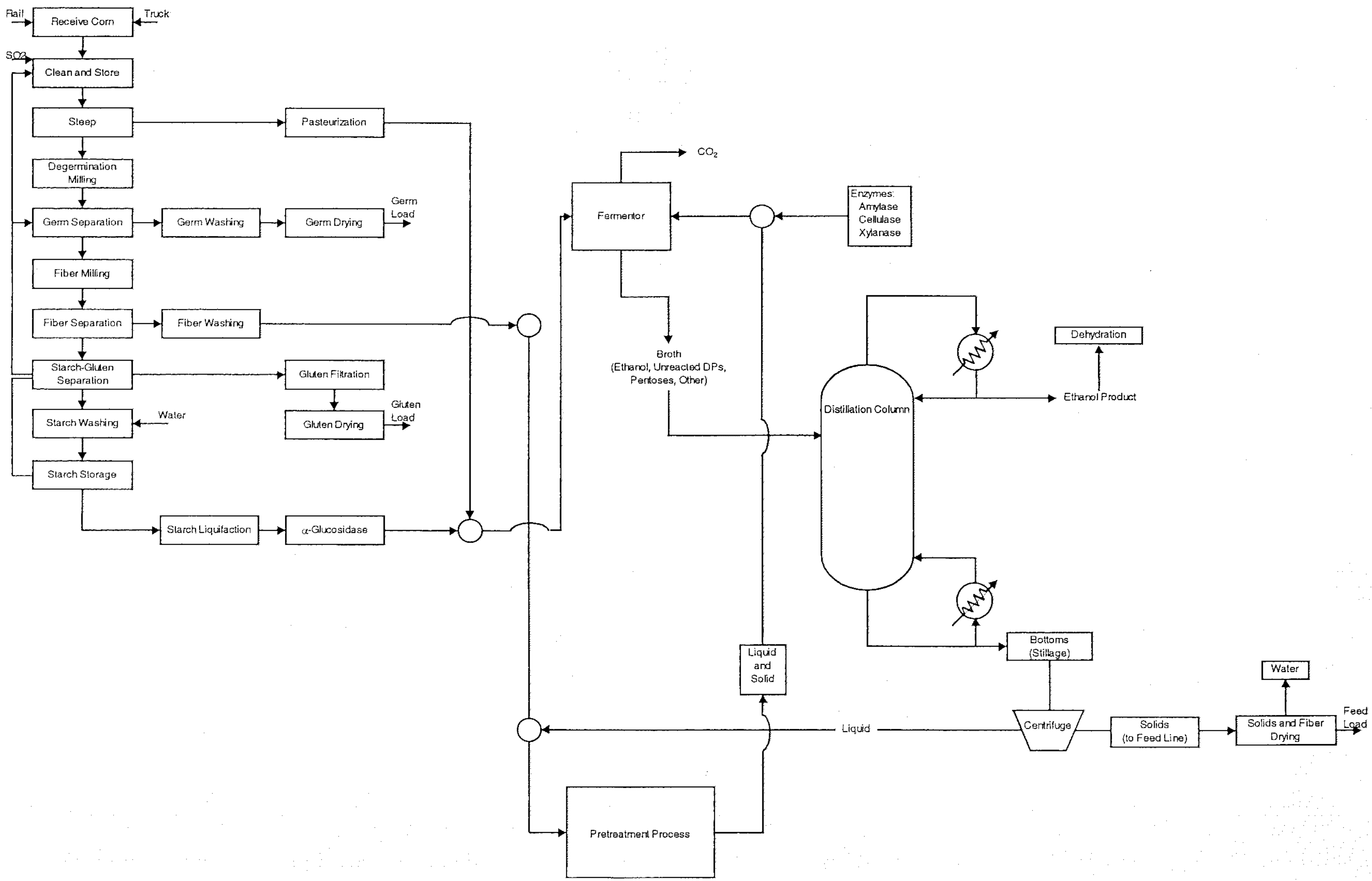


Figure 13: Flowsheet for Entire Pretreated Stream is Fermented

Table 7: Detailed List of Costs for Corn Fiber Entire Stream Case (95% hydrolysis of Theoretical and 90% Fermentation Efficiency)

FIBER VALUE ($65 Ethanol Cost ($/gallon)	All Fermentable Sugars	All Sugars Excluding Arabinose Ferment	Only Glucans, Glucose, Galactan, and DPs Ferment	Only Glucans, Glucose, and Galactan Ferment	Only Glucans and Glucose Ferment
Energy Cost for Pretreatment					
@ $5/10^6$ BTU	$0.0244	$0.0277	$0.0359	$0.0448	$0.0482
@ $2.5/10^6$ BTU	$0.0122	$0.0139	$0.0179	$0.0224	$0.0241
@ $1.8/10^6$ BTU	$0.0088	$0.0100	$0.0129	$0.0161	$0.0174
Capital Costs - New Plant	$0.0842	$0.0957	$0.1238	$0.1546	$0.1666
- Retrofit	$0.0732	$0.0833	$0.1077	$0.1345	$0.1449
Energy Cost for Distillation					
@ $5/10^6$ BTU	$0.0705	$0.0705	$0.0705	$0.0705	$0.0705
@ $2.5/10^6$ BTU	$0.0353	$0.0353	$0.0353	$0.0353	$0.0353
@ $1.8/10^6$ BTU	$0.0254	$0.0254	$0.0254	$0.0254	$0.0254
Net Fiber Cost	$0.4671	$0.5218	$0.5488	$0.5765	$0.5871
Enzyme costs	$0.0263	$0.0299	$0.0387	$0.0482	$0.0520
Reagent costs (chemicals)					
Labor costs					
Electricity costs					
Total Cost per Gallon with Steam Costs @ $5/MMBTU					
-New Plant Capital Costs	$0.6724	$0.7456	$0.8177	$0.8946	$0.9244
-Retrofit Capital Costs	$0.6615	$0.7332	$0.8016	$0.8745	$0.9028
@ $2.5/MMBTU					
-New Plant Capital Costs	$0.6250	$0.6965	$0.7645	$0.8370	$0.8650
-Retrofit Capital Costs	$0.6140	$0.6841	$0.7484	$0.8169	$0.8434
@ $1.8/MMBTU					
-New Plant Capital Costs	$0.6117	$0.6828	$0.7496	$0.8209	$0.8484
-Retrofit Capital Costs	$0.6007	$0.6703	$0.7335	$0.8008	$0.8268

Corn Fiber-Separate Enzyme Hydrolysis/Fermenter/Beer Still

This case is designed for a wet-milling facility that can only ferment liquids in the starch fermenters, therefore a separate enzyme hydrolysis/fermenter/beer still stream is used. The amount of incremental ethanol obtained from fiber and stillage in a 1000 lb_m/day plant inlet basis would be the same as for the case where the entire stream enters the starch fermenters (Table 6). The flowsheet for this case is found in Figure 14. The difference in this case from Figure 6 is that the pretreated fiber stream enters a separate fiber fermenter, beer well, and beer still. The ethanol vapor stream from the beer still is then sent to the rectifying column that is already present in the plant. The ethanol concentration from the fiber fermenters would be lower than 10%, if only glucose and hydrolyzed glucans and DPs are fermented. 10% ethanol is the concentration needed for economic distillation, so a glucose make-up stream from the starch hydrolysis tanks would be needed to raise the ethanol concentration to 10%. This glucose would be fermented in the starch fermenter to produce ethanol, so it is considered to have no value or cost.

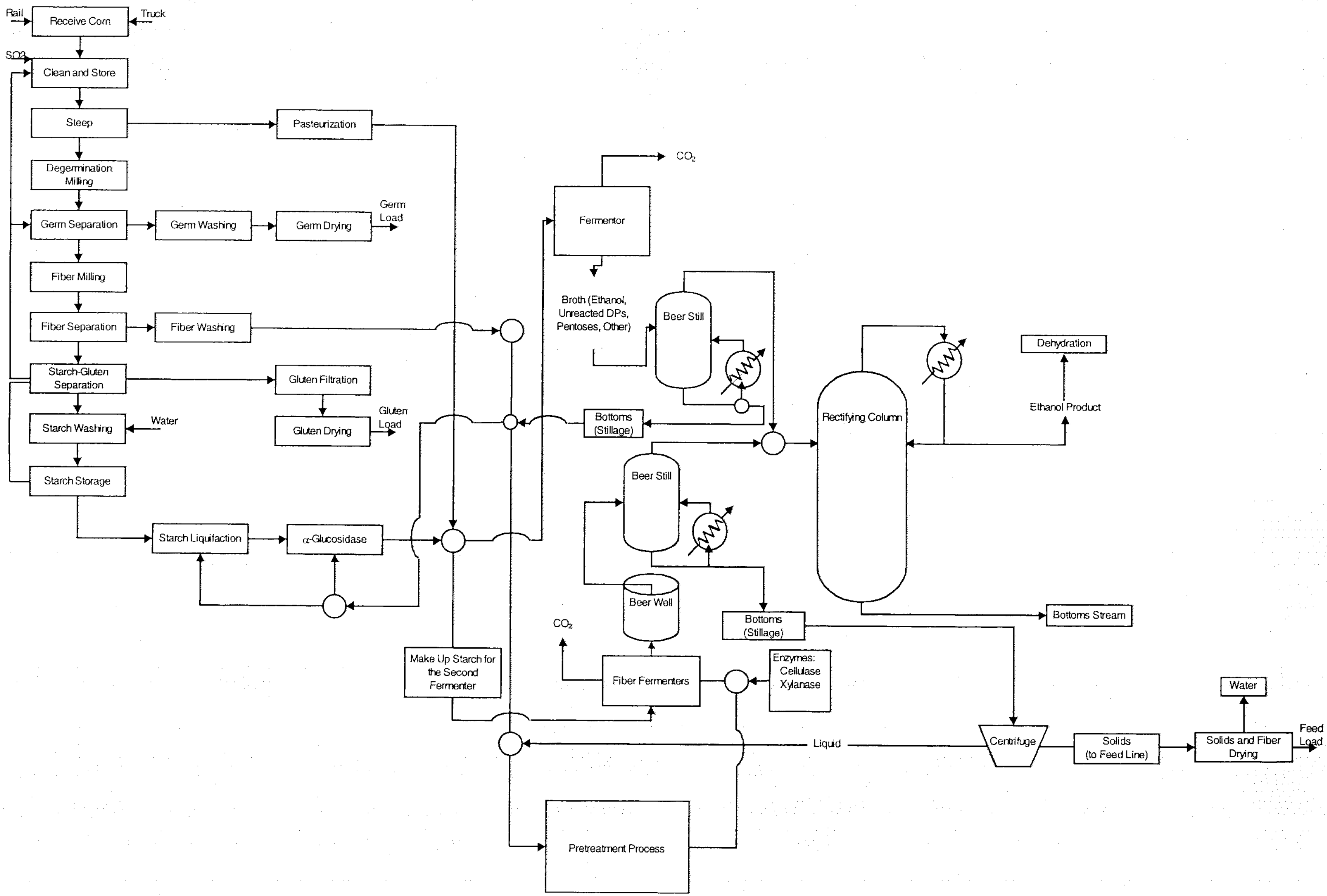


Figure 14: Flowsheet for a Separate Fiber Fermentation Stream

Table 8 shows the detailed costs for the separate fermentation/beer still. The prices per gallon for the third column are similar to the costs for the liquid-only fermentation, because even though more ethanol is being produced, the capital costs are substantially higher. The last two columns contain enzyme costs, because the solid glucans would need to be hydrolyzed, whereas before, there were no solid glucans in the liquid-only stream.

Table 8: Detailed List of Costs for Corn Fiber Separate Enzyme Hydrolysis/Fermenter/Beer Still (95% hydrolysis of Theoretical and 90% Fermentation Efficiency)

FIBER VALUE ($65) Ethanol Cost ($/gallon)	All Fermentable Sugars	All Sugars Excluding Arabinose Ferment	Only Glucans, Glucose, Galactan, and DPs Ferment	Only Glucans, Glucose, and Galactan Ferment	Only Glucans and Glucose Ferment
Energy Cost for Pretreatment					
@ $\$5/10^6$ BTU	$0.0244	$0.0277	$0.0359	$0.0448	$0.0482
@ $\$2.5/10^6$ BTU	$0.0122	$0.0139	$0.0179	$0.0224	$0.0241
@ $\$1.8/10^6$ BTU	$0.0088	$0.0100	$0.0129	$0.0161	$0.0174
Capital Costs - New Plant	$0.1290	$0.1467	$0.1898	$0.2369	$0.2553
- Retrofit	$0.1296	$0.1475	$0.1908	$0.2381	$0.2566
Energy Cost for Distillation					
@ $\$5/10^6$ BTU	$0.0705	$0.0705	$0.0705	$0.0705	$0.0705
@ $\$2.5/10^6$ BTU	$0.0353	$0.0353	$0.0353	$0.0353	$0.0353
@ $\$1.8/10^6$ BTU	$0.0254	$0.0254	$0.0254	$0.0254	$0.0254
Net Fiber Cost ($65/dry ton)	$0.4671	$0.5218	$0.5488	$0.5765	$0.5871
Enzyme costs	$0.0263	$0.0299	$0.0387	$0.0482	$0.0520
Reagent costs (chemicals)					
Labor costs					
Electricity costs					
Total Cost per Gallon with Steam Costs @ $5/MMBTU					
-New Plant Capital Costs	$0.7173	$0.7966	$0.8837	$0.9770	$1.0131
-Retrofit Capital Costs	$0.7179	$0.7974	$0.8847	$0.9782	$1.0144
@ $2.5/MMBTU					
-New Plant Capital Costs	$0.6698	$0.7475	$0.8305	$0.9193	$0.9538
-Retrofit Capital Costs	$0.6705	$0.7483	$0.8315	$0.9205	$0.9551
@ $1.8/MMBTU					
-New Plant Capital Costs	$0.6565	$0.7338	$0.8156	$0.9032	$0.9372
-Retrofit Capital Costs	$0.6572	$0.7345	$0.8166	$0.9044	$0.9384

Corn Stover

Corn Stover-15% Solids Loading

This case is for a corn stover inlet line to be separately ground, mixed with water so that there are 15% solids, pretreated at 180°C, hydrolyzed, fermented and sent through a beer still before the ethanol stream joins the ethanol from the starch fermenters. The amount of incremental ethanol obtained from a hypothetical 1000 lb_m/day plant inlet basis of corn (where the starch line would produce 51 gallons of ethanol/day) is shown in Table 9. The ratio of corn stover entering the plant to corn kernels entering the plant is small for this flowsheet, but the ratio can be increased depending on the plant design. The flowsheet for this case is shown in Figure 15. In this process, corn stover is shipped to the plant, and spray washed on a conveyor belt before being sent to the pretreatment system. The pretreatment process is the same as for the corn fiber. After being pretreated, the corn stover enters a separate set of fermenters, where the stover is enzymatically hydrolyzed and fermented to ethanol, and then the stream enters a beer well and beer still. Again, the concentrated ethanol vapors from the beer still enter the rectifying column already present in the plant. Also, a starch make-up stream would need to be diverted to raise the ethanol concentration to 10% if only glucose and hydrolyzed glucans and DPs were fermented.

In Table 9 the column headings are based on the sugars fermented. The three carbohydrates that are present are arabinan, xylan, and glucan. These carbohydrates are the polymers of arabinose, xylose, and glucose, respectively. For the first column, all three of the sugars would be fermented. For the second column only glucan and xylan would be fermented and for the third column, only glucan would be fermented.

Table 10 shows the detailed costs for the 15% solids-loading corn stover pretreatment stream. The 63% enzymatic hydrolysis is based on experimentation at Purdue University. The third column would be the most likely to be seen in industry currently, due to the probability that the first microorganism being used would most likely be yeast. The main cost is the substrate costs, but the corn stover could possibly have a large fuel value. If the stover could be burned for energy, it would have an estimated value of $28/dry ton based the 60% moisture coming out of the solid bowl centrifuge. If this were the case, then the cost of producing the ethanol would be close to the market value of ethanol. However, if xylose could be fermented, then the production of ethanol from corn stover could be profitable even without any residual value from the remaining corn stover.

Table 9: Incremental Ethanol from the Corn Stover at 15% Solids Loading Case for a hypothetical 1000 lb_m/day plant inlet basis of corn (where the starch line would produce 51 gallons of ethanol/day)

Total Ethanol Yield Assuming Fermentable Sugars Include:
(with 63% hydrolysis and 90% fermentation efficiency)

Total Ethanol Yield Assuming Fermentation of all Monosaccharides	0.621	gallons/day
Only Glucose and Xylan Fermentation	0.590	gallons/day
Only Glucose Fermentation	0.345	gallons/day

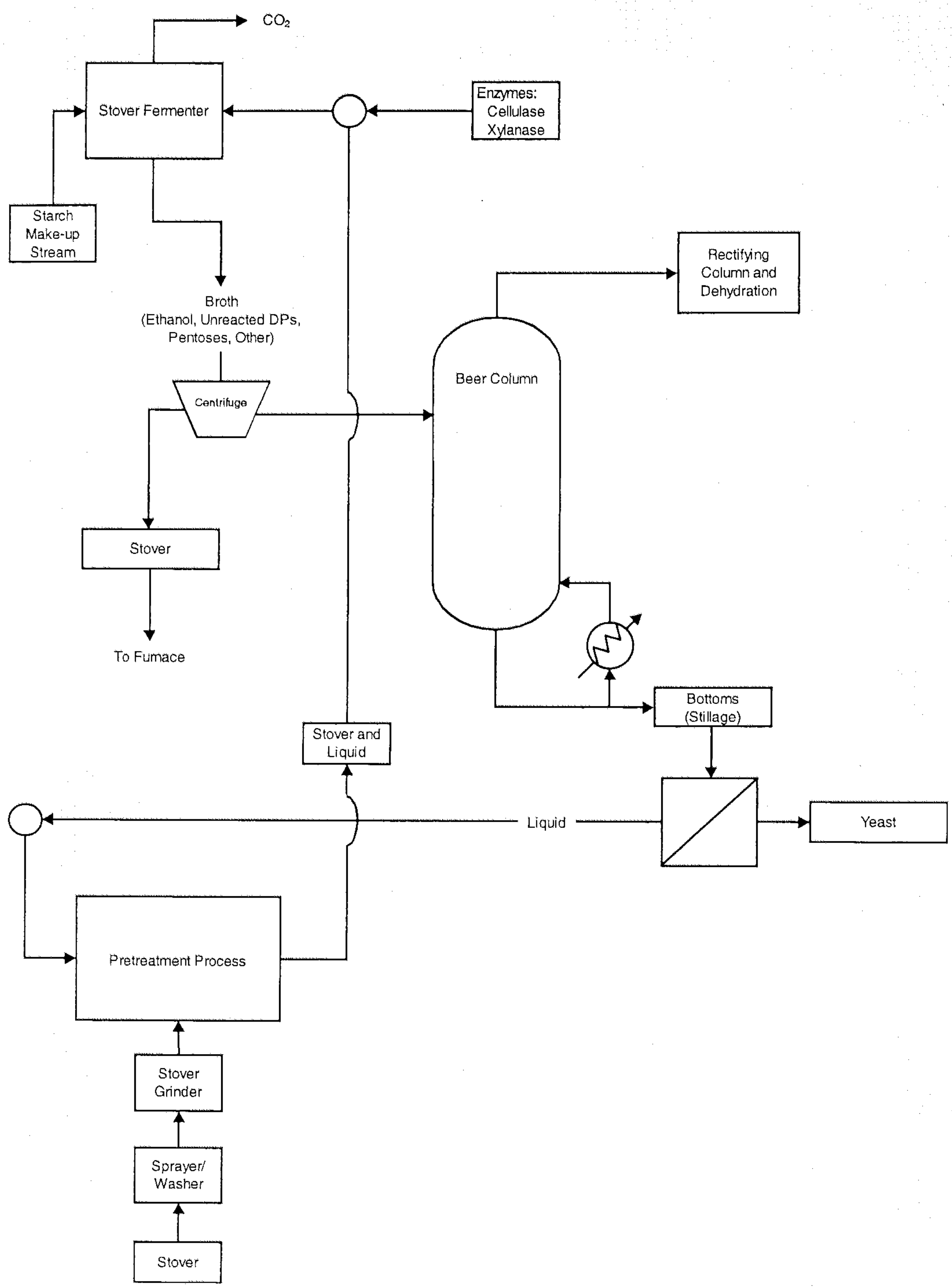


Figure 15: Flowsheet for the Corn Stover Pretreatment

Table 10: Detailed List of Costs for Corn Stover at 15% Solids Loading Case (63% hydrolysis of Theoretical and 90% Fermentation Efficiency)

FIBER VALUE ($65) Ethanol Cost ($/gallon)	All Fermentable Sugars	Only Hydrolyzed Glucans and Hydrolyzed Xylans	Only Hydrolyzed Glucans
Energy Cost for Pretreatment			
@ $5/10^6 BTU	$0.0338	$0.0355	$0.0607
@ $2.5/10^6 BTU	$0.0169	$0.0178	$0.0304
@ $1.8/10^6 BTU	$0.0122	$0.0128	$0.0219
Capital Costs - New Plant	$0.2326	$0.2449	$0.4185
- Retrofit	$0.1724	$0.1815	$0.3103
Energy Cost for Distillation			
@ $5/10^6 BTU	$0.0705	$0.0705	$0.0705
@ $2.5/10^6 BTU	$0.0353	$0.0353	$0.0353
@ $1.8/10^6 BTU	$0.0254	$0.0254	$0.0254
Net Stover Cost	$0.6144	$0.6469	$1.1057
Value @ $0/dry ton	$0.6144	$0.6469	$1.1057
Value @ $28/dry ton	$0.3845	$0.3982	$0.5915
Enzyme costs	$0.0493	$0.0519	$0.0886
Reagent costs (chemicals)			
Labor costs			
Electricity costs			
Total Cost per Gallon			
Value of Residual Solids @ $0/dry ton			
-New Plant Capital Cost			
@ $5/10^6 BTU	$1.0005	$1.0496	$1.7441
@ $2.5/10^6 BTU	$0.9483	$0.9966	$1.6784
@ $1.8/10^6 BTU	$0.9337	$0.9817	$1.6601
-Retrofitted Capital Cost			
@ $5/10^6 BTU	$0.9403	$0.9863	$1.6358
@ $2.5/10^6 BTU	$0.8882	$0.9333	$1.5702
@ $1.8/10^6 BTU	$0.8736	$0.9184	$1.5518
Value of Residual Solids @ $28/dry ton			
-New Plant Capital Cost			
@ $5/10^6 BTU	$0.7706	$0.8010	$1.2299
@ $2.5/10^6 BTU	$0.7185	$0.7479	$1.1643
@ $1.8/10^6 BTU	$0.7039	$0.7331	$1.1459
-Retrofitted Capital Cost			
@ $5/10^6 BTU	$0.7105	$0.7376	$1.1217
@ $2.5/10^6 BTU	$0.6583	$0.6846	$1.0560
@ $1.8/10^6 BTU	$0.6437	$0.6698	$1.0377

Corn Stover-40% Solids Loading

This case is for a corn stover inlet line to be separately ground, mixed with water so that there are 40% solids, pretreated at 180°C, hydrolyzed, fermented and sent through a beer still before the ethanol stream joins the ethanol from the starch fermenters. The amount of incremental ethanol obtained from a hypothetical 1000 lb_m/day plant inlet basis of corn (where the starch line would produce 51 gallons of ethanol/day) is shown in Table 11. The flowsheet and process for this case is the same as for the corn stover 15% solids loading. A starch make-up stream would need to be added to raise the output ethanol concentration to 10%, unless xylose was fermented, in which case, no starch make-up stream would be needed.

Table 12 shows the detailed costs from 40% solids corn stover loading. The substrate cost is the most significant cost, but the amount of ethanol produced will be much greater than in the 15% loading case. However, the 40% loading would have significant solids handling issues that would need to be overcome, due to the high viscosity. Again, the process could be economically feasible if either the residual corn stover could be burned as fuel or if the xylose could be fermented.

Table 11: Incremental Ethanol from the Corn Stover at 40% Solids Loading Case from a hypothetical 1000 lb_m/day plant inlet basis of corn (where the starch line would produce 51 gallons of ethanol/day)

Total Ethanol Yield Assuming Fermentable Sugars Include:
(with 63% hydrolysis and 90% fermentation efficiency)

Total Ethanol Yield Assuming Fermentation of all Monosaccharides	1.65 gallons/day
Only Glucose and Xylan Fermentation	1.57 gallons/day
Only Glucose Fermentation	0.92 gallons/day

Table 12: Detailed List of Costs for Corn Stover at 40% Solids Loading Case (63% hydrolysis of Theoretical and 90% Fermentation Efficiency)

FIBER VALUE ($65) Ethanol Cost ($/gallon)	All Fermentable Sugars	Only Hydrolyzed Glucans and Hydrolyzed Xylans	Only Hydrolyzed Glucans
Energy Cost for Pretreatment			
@ $5/10^6 BTU	$0.0127	$0.0134	$0.0228
@ $2.5/10^6 BTU	$0.0063	$0.0067	$0.0114
@ $1.8/10^6 BTU	$0.0046	$0.0048	$0.0082
Capital Costs - New Plant	$0.0874	$0.0920	$0.1573
- Retrofit	$0.0648	$0.0682	$0.1166
Energy Cost for Distillation			
@ $5/10^6 BTU	$0.0705	$0.0705	$0.0705
@ $2.5/10^6 BTU	$0.0353	$0.0353	$0.0353
@ $1.8/10^6 BTU	$0.0254	$0.0254	$0.0254
Net Stover Cost			
Value @ $0/dry ton	$0.6146	$0.6471	$1.1060
Value @ $28/dry ton	$0.3847	$0.3984	$0.5919
Enzyme costs	$0.0493	$0.0519	$0.0886
Reagent costs (chemicals)			
Labor costs			
Electricity costs			
Total Cost per Gallon			
Value of Residual Solids @ $0/dry ton			
-New Plant Capital Cost			
@ $5/10^6 BTU	$0.8344	$0.8748	$1.4452
@ $2.5/10^6 BTU	$0.7928	$0.8329	$1.3986
@ $1.8/10^6 BTU	$0.7812	$0.8211	$1.3855
-Retrofitted Capital Cost			
@ $5/10^6 BTU	$0.8118	$0.8510	$1.4046
@ $2.5/10^6 BTU	$0.7702	$0.8091	$1.3579
@ $1.8/10^6 BTU	$0.7586	$0.7973	$1.3448
Value of Residual Solids @ $28/dry ton			
-New Plant Capital Cost			
@ $5/10^6 BTU	$0.6046	$0.6261	$0.9311
@ $2.5/10^6 BTU	$0.5630	$0.5842	$0.8844
@ $1.8/10^6 BTU	$0.5513	$0.5725	$0.8714
-Retrofitted Capital Cost			
@ $5/10^6 BTU	$0.5820	$0.6023	$0.8904
@ $2.5/10^6 BTU	$0.5404	$0.5604	$0.8438
@ $1.8/10^6 BTU	$0.5287	$0.5487	$0.8307

Conclusions

Corn Fiber

The corn fiber pretreatment process consisting of pretreatment/ enzyme hydrolysis/ fermentation can currently be used to make an economically viable process even if only glucose and hydrolyzed glucans and DPs are fermented to ethanol. The production price will be between \$0.77 and \$0.87 per gallon not including labor, facility electricity and reagents.

Corn Stover

The corn stover pretreatment process is very close to being economically feasible, and it depends on either being able to convert the xylose as well as the glucose, or being able to burn the remaining corn stover residue for fuel. However, more experimentation is also needed for the corn stover hydrolysis.

Appendix: Fermentation experiments on pretreated corn fiber run at USDA by Bruce Dien.

NOTE: The corn fiber hydrolysate (CFH) is actually just pretreated corn fiber without any enzymatic hydrolysis. It is referred to as hydrolysate in keeping with terminology that is commonly used in pretreatment literature. However, the pretreatment process for corn fiber has actually been optimized to minimize hydrolysis to monosaccharides during the pretreatment itself. Hence, there are only very small amounts of monosaccharides prior to enzyme hydrolysis.

Introduction

Shake flask fermentations were run using William Energy Service's corn starch hydrolysate, light corn steep liquor, and well water. Each of these was taken right off the production line and added in the recommended proportion. Alltech's yeast was used for the inoculum at $2x10^7$ cells/ml. The corn fiber hydrolysate was used as received from Purdue; it was not further hydrolyzed with enzymes. All solutions were filtered sterilized, except the well water, which was autoclaved. Two experiments were run. In the first, corn fiber hydrolysate (CFH) was substituted for either none, half, or all of the corn steep liquor (CSL). In the second, CFH was substituted for either none, half, or all of the well water. Each treatment was run in duplicate. The shake flasks, used for the fermentations, were equipped with alwood valves -- these were filled with concentrated sulfuric acid and trap all volatiles except carbon dioxide. The progress of the fermentation was tracked by monitoring weight loss, which is correlated with carbon dioxide production. The flasks were analyzed for initial and residual sugar and ethanol production by HPLC.

Conclusions

Addition of hot water treated corn fiber hydrolysate appears to have no significant effect when used to replace either the corn steep liquor or well water (probability of significance for F test > 0.56 for each). Based upon visual inspection of the plots, however, substitution of the corn fiber hydrolysate for 100% v/v of the corn steep liquor does appear to slow yeast fermentations. This slowing might be nutrition related because the effect was not observed when 100% of the well water was replaced. However, this amount of corn fiber hydrolysate far exceeds the concentration to be added as specified by the mass balance and was run as an added sensitivity check. Also note, that in as far as the corn fiber hydrolysate used was not treated with enzymes, it would not have been a source of significant glucose to the fermentation.

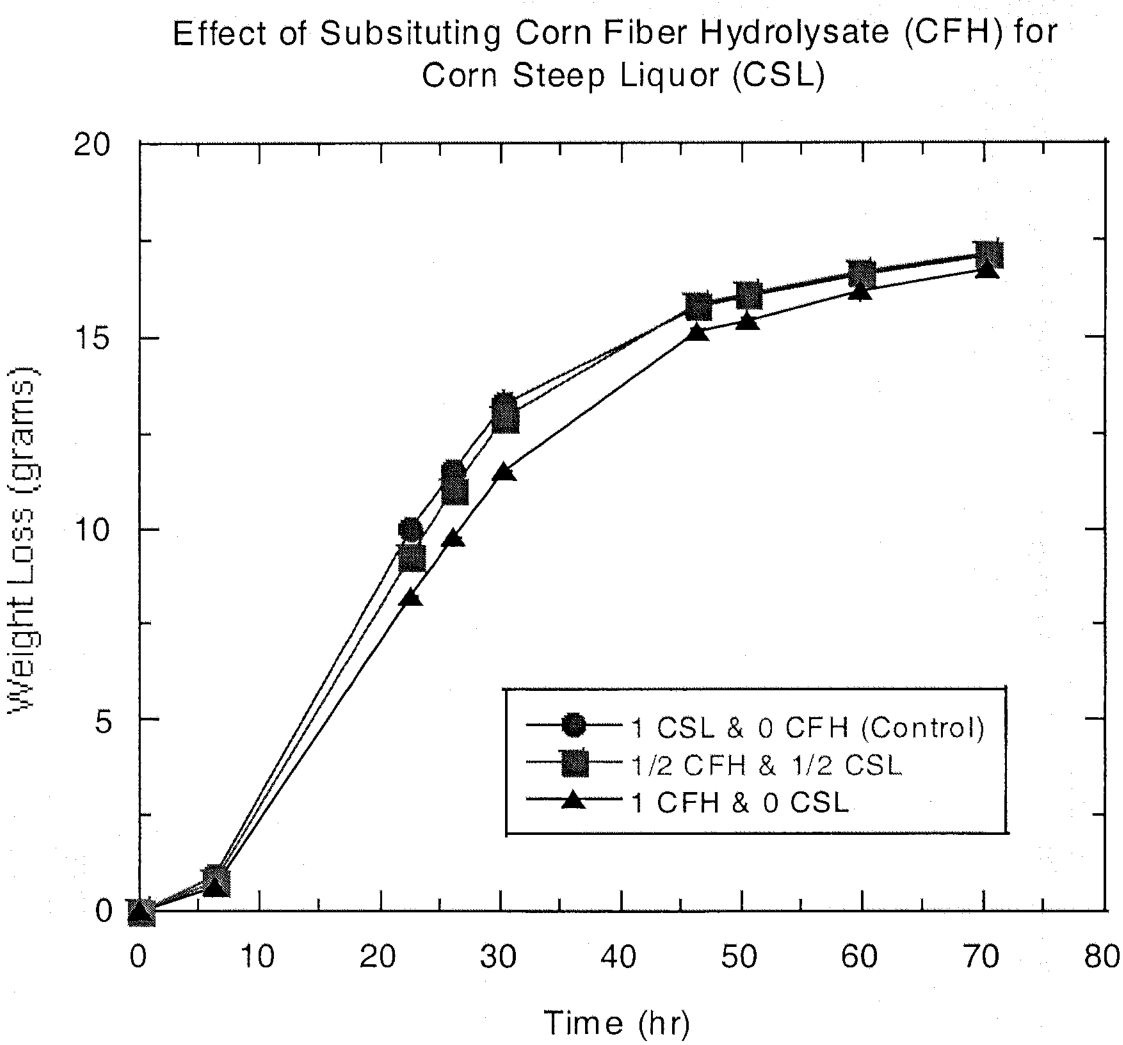
Results

Purdue Hydrolysate %v/v	Corn-Steep Liquor %v/v	Well Water %v/v	Residual Glucose %w/v	Final Ethanol %v/v	Metabolic Yield g/g	Production Yield g/g	Marginal Production Yield %
Control							
0%	100%	100%	0.65	11.2	0.50	0.48	0.0
Replacing Corn steep with hot water hydrolysate							
50%	50%	100%	0.59	11.2	0.50	0.48	0.4
100%	0%	100%	0.96	11.0	0.50	0.47	-1.7
Replacing well water with hot water hydrolysate							
50%	100%	50%	0.59	11.0	0.49	0.47	-1.7
100%	100%	0%	0.70	11.3	0.50	0.48	0.8

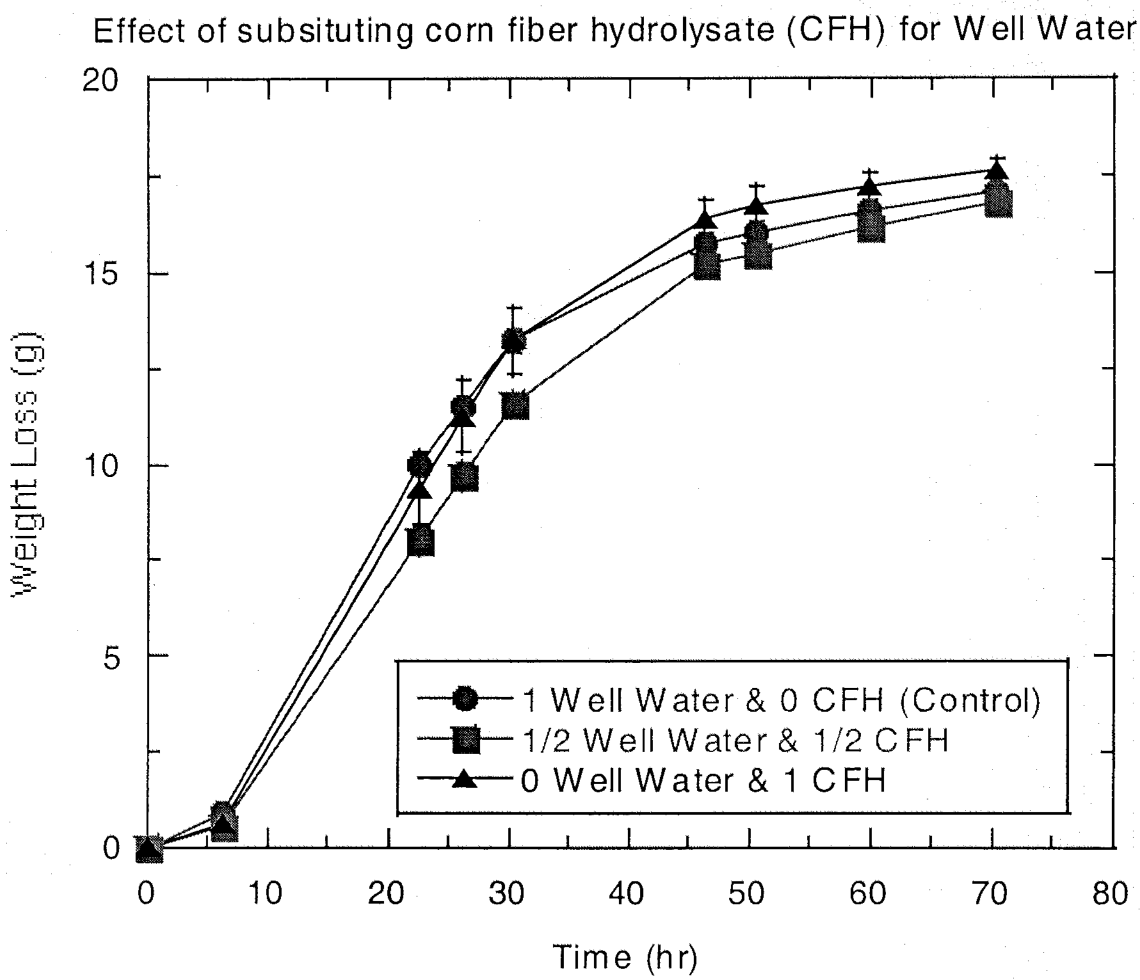
Notes for Table:

1. All calculation of the average of duplicate runs.
2. The first three columns describe the media preparations. Either 50%v/v or 100%v/v of the well water and corn steep liquor was replaced with the corn fiber hot water treated hydrolysate.
3. The residual glucose was measured after 72 hr. In a previous experiment, to test out the system, all the glucose was fermented. However, the corn starch hydrolysate contained a lower concentration of sugar.
4. The metabolic yield is the ratio of the amount of ethanol produced and the amount of glucose consumed. Theoretical is 0.51 g/g.
5. The production yield is the ratio of the amount of ethanol produced and the amount of glucose added (i.e. residual glucose is not factored into the calculation).
6. The marginal process yield is the % difference between the given treatment and the control.

Analysis: substitution of 1/2 of the CSL with CFH does not appear to effect on ethanol productivity. I doubt if substituting 1/2 of the CSL with well water will be significant either. Error bars are included on plots shown below.



Effect of Subsituting Corn Fiber Hydrolysate (CFH) for Corn Steep Liquor (CSL)
20
15
10
5
0
0
10
20
30
40
50
60
70
80
Weight Loss (grams)
Time (hr)
1 CSL & 0 CFH (Control)
1/2 CFH & 1/2 CSL
1 CFH & 0 CSL



Introduction: Corn fiber hydrolysate (cfh), supplied directly from Purdue, was fermented to ethanol using two ethanologenic *E. coli* strains. Fermentation of hydrolysates can be problematic because they often contain side-products formed during hydrolysis that inhibit microbial growth. This set of experiments was preformed to determine if an additional pretreatment step is needed to remove the inhibitors. The reader should note that cfh was not treated with enzymes to convert the oligomers into fermentable monomers. The experiments focused on measuring inhibition effects.

The two strains tested were *E. coli* FBR5 and K011. Both strains are recombinant *E. coli* that are capable of selectively converting the sugars present in cfh (i.e. arabinose, galactose, glucose, and xylose) to ethanol. *E. coli* strain K011 was constructed in Dr Ingram's laboratory (University of Florida). *E. coli* strain FBR5 was developed by our laboratory. In a previous memo, we reported that *E. coli* strain K011 would not ferment cfh. In this study, the seed culture was pre-adapted to cfh.

Conclusions: Both strains were sensitive to the presence of cfh, though *E. coli* strain FBR5 appears less sensitive than *E. coli* strain K011. Pre-adaptation of the seed culture of *E. coli* K011 had no effect on its ability to ferment cfh. Future efforts will focus on fermenting cfh that has been treated to remove inhibitors according to a protocol developed by Dr. Ladisch's laboratory, which has been shown to be effective.

Results (all results are average of duplicate runs):

1. Determination of minimum inhibitor concentration (%v/v) for *E. coli* strain K011 (Fig. 1)

Seed cultures of *E. coli* K011 were grown on LB medium supplemented with 5% w/v xylose and varying concentrations of cfh (0 - 50%v/v). The cultures were grown micro-aerophilic at 30°C. After 24 hr, cell growth and residual xylose were measured. Cfh concentrations greater than 10% v/v inhibited sugar consumption by more than 50%. Therefore, for the next experiment, the seed culture medium was supplemented with 10% v/v cfh.

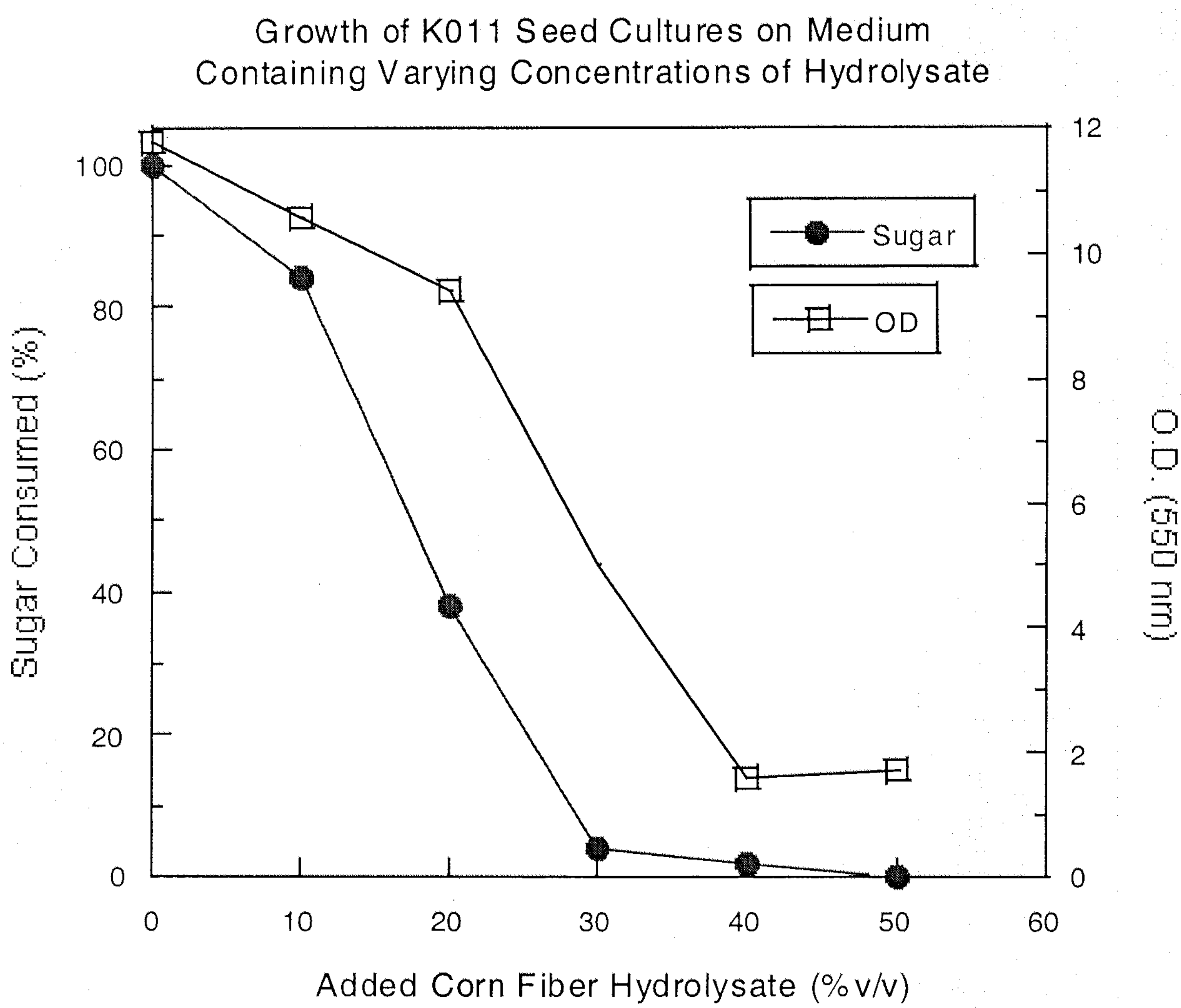
2. Fermentation of CFH with an adapted *E. coli* K011 seed culture (Fig. 2)

Seed cultures were grown in the presence of cfh (10% v/v). These seed cultures were used to inoculate cfh (90%v/v) supplemented with tryptone and yeast extract and Pipes buffer (pH = 7; 0.1 M) or a mixed sugar control with similar supplements. Additional controls included inoculating cfh and mixed sugar cultures from a seed culture not pre-adapted. The flasks were capped with rubber closures and incubated at 35°C. Sugar and ethanol concentrations were measured daily. Pre-adaptation of *E. coli* K011 neither aided the cfh fermentation nor hindered the mixed sugar fermentation based upon ethanol yield.

3. Determination of minimum inhibitor concentration (%v/v) for *E. coli* strain FBR5 (Fig. 3)

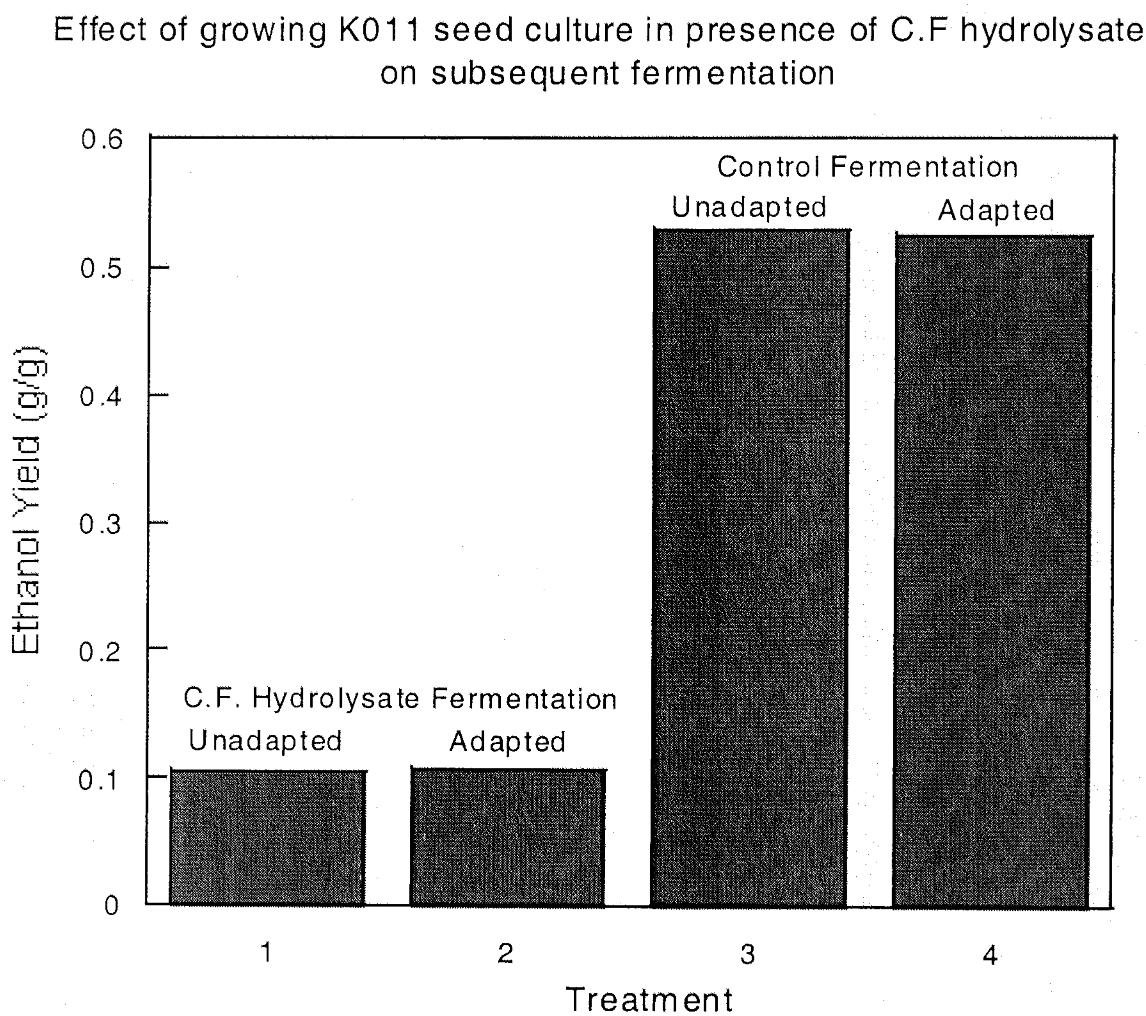
Seed cultures of *E. coli* FBR5 were cultured in anaerobic LB medium supplemented with 4 g/l xylose and 0 - 40% v/v cfh. The cultures were incubated at 37°C for 12 hr before sampling for cell yield and residual xylose. The results demonstrate that cfh concentrations greater than 20% v/v inhibit sugar consumption by greater than 50%.

Figure 1



Growth of K011 Seed Cultures on Medium
Containing Varying Concentrations of Hydrolysate
Sugar
OD
Sugar Consumed (%)
O.D. (550 nm)
Added Corn Fiber Hydrolysate (% v/v)
0
20
40
60
80
100
0
2
4
6
8
10
12
0
10
20
30
40
50
60

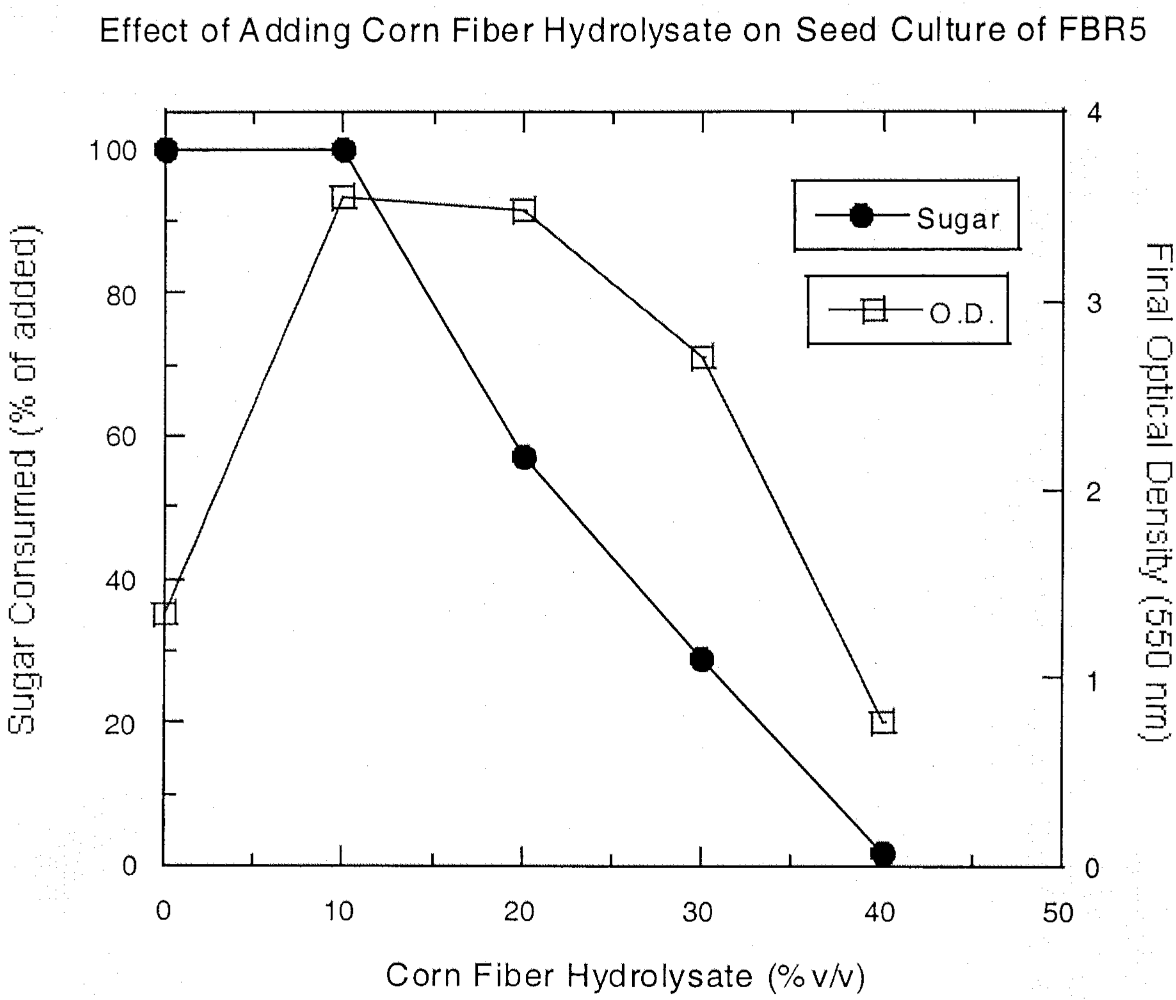
Figure 2



Description of Treatments

Treatment	Seed Culture	Fermentation
1	Not adapted	CFH
2	Adapted to CFH	CFH
3	Not adapted	Mixed sugars
4	Adapted to CFH	Mixed sugars

Figure 3



Effect of Adding Corn Fiber Hydrolysate on Seed Culture of FBR5
Sugar
O.D.
Sugar Consumed (% of added)
Final Optical Density (550 nm)
Corn Fiber Hydrolysate (% v/v)
0
20
40
60
80
100
0
1
2
3
4
0
10
20
30
40
50